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From the Children's Hospital, University of Helsinki, Finland

# Malabsorption Syndrome in Childhood

## *The Occurrence of Absorption Defects and Their Clinical Significance*

*by* J. K. VISAKORPI, PIRKKO IMMONEN and P. KUITUNEN

Defective intestinal absorption is one of the most characteristic findings in coeliac disease. Beside the determination of faecal fat, many new tests have been developed for the assessment of intestinal absorption. Among these, the D-xylose excretion test and the determination of urinary FIGLU have come into wide clinical use. Although these tests usually enable a clear differentiation to be made between normals and patients with coeliac disease, abnormal results may be obtained in many other diseases and clinical states, which can all be listed under the title malabsorption syndrome. Furthermore, these indirect tests may give "false positive" results for various reasons [*, 3]. Therefore evaluation of the significance of the results of these absorption tests is difficult and has rarely been carried out in a series of patients suffering from different forms of the malabsorption syndrome.

In this study absorption defects have been searched for by means of faecal fat determination, the D-xylose excretion test and the FIGLU test in children with symptoms suggestive of the malabsorption syndrome. The diagnostic significance of these tests has been evaluated by comparing the results with the final clinical diagnoses of the patients and with the duodenojejunal histology.

### Material

The patients studied were all treated at the Children's Hospital, University of Helsinki, during the three-year period 1962–65. During that period every patient suspected of having the malabsorption syndrome was included in this study. Suspicion of the malabsorption syndrome was based on the following symptoms: abnormal stools for more than 2 weeks (diarrhoea or otherwise abnormal stools), failure to gain weight and failure to grow. These base symptoms usually occurred together, but patients with one of them were also included. The patients had also other symptoms, but the selection was based on the abovementioned. The number of patients and the age distribution are presented in Table 1 and Fig. 1.

Every patient included in the study was subjected to a similar series of absorption tests, comprising faecal fat determination, the D-xylose excretion test and the FIGLU test. Thereafter a peroral small bowel biopsy was attempted. It was not possible to carry out all the tests in every case. The final clinical diagnosis was reached by making other diagnostic tests, such as barium

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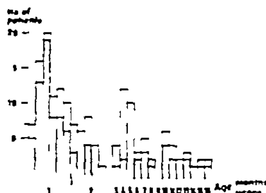


FIG. 1. Age distribution of the patients studied. The shaded area of the columns indicates patients with absorption defects.

meal, sweat test d saccharide loading test and measurement of pancreatic enzymes. The patient w th bsorption defect without known cause were then subm tted to a therapeutic trial with limination diet. The patient were at first treated w th their regular diets for at least weeks, after which, if no signs of improvement were observable a gl ten free diet was instituted. If there was no improvement on this regimen, a diet without cow milk was started (breast milk). In about half the cases an elimination diet was instituted soon after admission, and the intolerance was erified by provocation of ymptoms when the patient was clearly improving.

The patients ha e been followed up from 6 months to 3 years.

Four patients were either discharged before completion of the diagnostic studies or lost to follow up. The final linical diagnosis remained open in these cases.

## Method

Faecal f t was measured chemically according to the method f van de Kamer *et al.* [8]. The faeces corresponding to 3 days intak on the patient regular diet were collected using carmine as marker. The a erage daily excretion was computed. The intake was also calculated but resorption indices will not be discussed in this paper because calculations of intake ppeared to be inaccurat.

The D x lose test was performed by collecting 5 hours urine on good diuresis (more than 100 ml urine) after a single oral load of 350 mg D-xylose per kg body weight after an eight hour fast. Urinary D-xylose was measured by the method described in the manual of O'Brien & Ibbot [1].

Urinary FICLU was measured by high voltage lectrophoresis according to Knowles [9] from 1 -hour urine after a single 1-histidine load (350 mg per kg body weight).

Detail of the biopsy technique ha e been described by Kuitunen [10].

TABLE 1. *Symptoms and igns of pat ent with and without absorption defects*

| | No. of patient with absorption defect | No. of patient without absorption defects | Total |
|---|---|---|---|
| No. of patients | 88 | 65 | 153 |
| *Main symptoms* | | | |
| Abnormal stools and failure t gain weight or to grow | 67 | 23 | 90 |
| Abnormal stool only | 11 | 23 | 34 |
| F ilure to gain weight and/or to grow only | 10 | 19 | 29 |
| *Additional symptoms and signs* | | | |
| Vomiting, bdominal discomfort | 81 | 28 | 79 |
| Bleeding oedema, tetany | 7 | 0 | 7 |
| Bulging bdomen | 46 | 3 | 49 |

The diagnostic tests for disaccharide mal absorption were performed as described by Launiala *et al* [11].

## Definitions of Clinical Diagnoses

Patients with increased faecal fat and/or abnormal D-xylose excretion were listed under the title of beorption defects. In addition, patients, in whom disaccharide malabsorption was verified with special tests were included in this group.

The normal value of faecal fat was taken as less than 4 g per day in infants and less than 5 g per day in older children. These alues approximately correspond to the 85–90 % normal limit f the resorption index est blished by Weijers & van de Kamer [14]. The normal excretion in the D xylose test was considered to be 15 % or more of the ingested doses according to the studies of H bble & Littlejohn [5] and Hadorn *et al* [4].

Primary malabsorption syndrome: This group includes those patients with absorption defects who xhibited clinical intolerance to gl ten or cow milk [15]. Many of the patients with cow milk intolerance were also intolerant to gluten.

Secondary malabsorption syndrome: In these patients, the presence of another disease which causes malabsorption or is often accompanied by malabsorption, was verified.

Chronic malabsorption syndrome of un known aetiology: Patient in whom no response t elimination diets was observed.

Transient absorption defects: Patients who recovered during the observ tion period without any special treatment

## Results

### *The occurrence of absorption defects*

Among the 153 children in whom mal absorption was suspected defective absorption was found in 83 cases. Fig. 1 shows the age distribution of these patients Most of the patients studied were infants and the occurrence of absorption defect was particularly high among the young infants.

### *Symptoms and signs connected with absorption defects*

Table 1 shows that the combined symptomatology of both abnormal stools and failure to gain was the best indication of absorption defects, although it was not pathognomonic. Absorption defects were also found in monosymptomatic patients having only abnormal stools or failure to gain. Some of the dditional symptoms and signs, such as vomiting were non specific whereas bleeding oedema and tetany were specific but rare Bulging abdomen was also seen more frequently in the group where absorption defect was verified.

### *Cl ical diagnoses*

The final clinical diagnoses are presented in Table * Of the patients with beorption defects 57 % (48) proved to have the primary malabsorption syndrome with intolerance to gluten and/or to cow s milk. In 23 % (19) of the cases the malabsorption syndrome was secondary In 20 % (17) no specific aetiology for the absorption defect was found. In six of the last men tioned patients the disease was long standing and severe but no food intoler ance was observable Three of these six also had Down s syndrome. Chronic enteric infection is a possible explanation of the absorption defect in these cases. Eleven patients had only transient absorption defects in connection with prolonged use of antibiotics acut infections or other diseases

The patients with normal absorption

TABLE 2. *Final clinical diagnoses*

| Diagnosis | No. of patients | | |
|---|---|---|---|
| | Infants | Over 2 years | Total |
| *Patients with absorption defects* | 66 | 22 | 88 |
| Primary malabsorption syndrome | 40 | 8 | 48 |
| cow's milk intolerance | 12 | 0 | 12 |
| gluten intolerance | 28 | 8 | 36 |
| Secondary malabsorption syndrome | 10 | 9 | 19 |
| pancreatic insufficiency[a] | 3 | 3 | 6 |
| disaccharide malabsorption | 5 | 0 | 5 |
| small bowel resection | 1 | 0 | 1 |
| malabsorption syndrome in systemic diseases[b] | 1 | 6 | 7 |
| Chronic malabsorption syndrome of unknown aetiology (no intolerance) | 6 | 0 | 6 |
| Transient absorption defects | 9 | 2 | 11 |
| Discharged before completion of diagnostic studies | 1 | 3 | 4 |
| *Patients without absorption defects* | 34 | 29 | 63 |

[a] Cystic fibrosis 4, pancreatic achylia 2.
[b] Diabetes mellitus 4, hypoparathyroidism 1, cirrhosis of the liver 1 and ulcerative colitis 1.

test results proved to have diarrhoea of infectious origin, ulcerative colitis and failure to gain weight or failure to grow caused by systemic diseases. None of these has subsequently developed malabsorption syndrome.

*Correlation of absorption test results and clinical diagnoses*

The results presented in Table 3 demonstrate that increased faecal fat was the commonest finding. Fig. 2 shows that there was no clear difference between high-normal and low-abnormal values. Very high faecal fat values were constantly measured in pancreatic insufficiency. Medium high values could often be seen in the primary malabsorption syndrome. In the last-mentioned group normal values were rare, and in every case were due to low intake of fat. In the two non-specific groups, namely in patients with chronic malabsorption syndrome of unknown aetiology and in those with transient absorption defects, faecal fat excretion was usually only slightly elevated.

Abnormally low D-xylose excretion was a rather less common finding, which was obtained especially in primary malabsorption (Table 3). Again, no clear boundary between normal and pathological values could be seen (Fig. 3). When the excretion of 15% of the test dose was choosen as the limit between normal and pathological values, normal results were obtained for one-third of the patients with the primary malabsorption syndrome. If 10% had been taken as the limit, the result would have been normal in less than 10% (4) of these patients, but at

| Diagnoses | Fat + | Fat − | Xylose + | Xylose − | FIGLU + | FIGLU − | Fat Xylose + + | Fat Xylose + − | Fat Xylose − + | Fat Xylose − − | F t, Xylose, FIGLU + + + | + − + | − + + | − − + | + + − | + − − | − + − | − − − |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Patients with absorption defects* | 44 | 3 | 33 | 13 | 39 | 5 | 29 | 13 | 3 | 0 | 26 | 11 | 3 | 0 | 3 | 3 | 0 | 0 |
| Primary malabsorption syndrome | 11 | 1 | 7 | 4 | 8 | 2 | 6 | 4 | 1 | 0 | 6 | 3 | 0 | 0 | 0 | 3 | 0 | 0 |
| cow's milk intolerance | 33 | 2 | 26 | 9 | 31 | 3 | 23 | 9 | 3 | 0 | 20 | 9 | 2 | 0 | 3 | 0 | 0 | 0 |
| gluten intolerance | 16 | 3 | 4 | 15 | 4 | 12 | 2 | 14 | 2 | 1 | 0 | 3 | 1 | 0 | 1 | 11 | 0 | 0 |
| Secondary malabsorption syndrome | 6 | 0 | 0 | 6 | 0 | 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 |
| pancreatic insufficiency | 2 | 3 | 3 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| disaccharide malabsorption | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| small bowel resection | | | | | | | | | | | | | | | | | | |
| malabsorption syndrome in systemic diseases | 7 | 0 | 0 | 7 | 3 | 4 | 0 | 7 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 4 | 0 | 0 |
| Chronic malabsorption syndrome of unknown aetiology (no intolerance) | 4 | 2 | 5 | 1 | 3 | 3 | 3 | 1 | 2 | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 1 | 0 |
| Transient absorption defects | 8 | 3 | 5 | 6 | 5 | 3 | 2 | 6 | 3 | 0 | 0 | 3 | 2 | 8 | 0 | 2 | 1 | 0 |
| *Patients without absorption defects* | 0 | 65 | 0 | 65 | 8 | 45 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 45 |

\+ abnormal test result.
− normal test result.

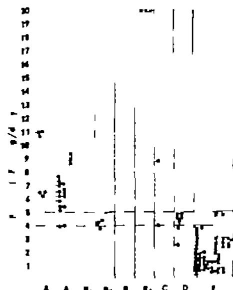



Fig 2. Faecal fat excretion (g per da ) in the different clinical groups.

*A* Primary malabsorption syndrome; *1* cow milk intolerance; *2*, gluten intolerance *B* Secondary malabsorption syndrome; *1* pancreatic insufficiency; *2*, disaccharide malabsorption; *3*, small bowel resection; *4* malabsorption syndrome in systemic diseases. *C* Chronic malabsorption syndrome of unknown aetiology *D* Transient absorption defects. *E* Patients without absorption defects. ● Infants. − Children.

the same time the number of pathological xylose tests would have increased considerably among patients without other absorption defects, especially among the infants (Fig 3)

A positive FIGLU test was found with about the same frequency and in the same groups as abnormal xylose excretion (Table 3). 15% of the patients without other absorption defects had detectable amounts of FIGLU in the urine

The combined results of the absorption tests are also presented in Table 3. When both faecal fat and D xylose excretion were abnormal, the diagnosis of th primary malabsorption syndrome was ap-

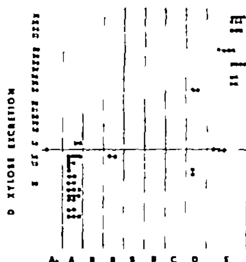


Fig. 3. D-xylose excretion in different clinical groups. Explanations as in Fig. —

parent and when, in addition the FIGLU test was positive this diagnosis was almost certain. Of 44 patients with the primary malabsorption syndrome 4 had at least two of these three tests abnormal. The pattern of abnormal faecal fat and normal D-xylose FIGLU was typical of pancreatic insufficiency but was also fairly often encountered in other groups especially in malabsorption syndrome associated with systemic diseases.

*Duodenojejunal histology*

Table 4 shows that more or less severe histological changes of the small intestinal mucosa were usually observed in patients with absorption defects except in those in whom the defect was due to digestive failure. Severe villous atrophy was a typical finding in primary malabsorption.

*Correlation of absorption tests with duodenojejunal histology*

Table 5 shows that abnormal test results pointed to damaged intestinal mucosa

TABLE 4. *Duodenojejunal histology in various clinical groups*

| Diagnosis | No. of patients | Duodenojejunal histology: Normal mucosa | Slight changes | Partial atrophy | Subtotal atrophy |
|---|---|---|---|---|---|
| *Patients with absorption defects* | 48 | 8 | 9 | 9 | [illegible] |
| Primary malabsorption syndrome | 30 | 1 | 2 | | 20 |
| cow's milk intolerance | 10 | | 2 | 3 | 5 |
| gluten intolerance | 20 | 1 | | 4 | 15 |
| Sec. malabsorption syndrome | 10 | 6 | | 1 | 1 |
| pancreatic insufficiency | | 2 | | | |
| disaccharide malabsorption | 4 | 3 | | 1 | |
| small bowel resection | 1 | 1 | | | |
| malabsorption syndrome in systemic diseases | 3 | | [illegible] | | 1 |
| Chronic malabsorption syndrome of unknown aetiology | 4 | | 3 | 1 | |
| Transient absorption defects | 3 | 1 | 1 | | 1 |
| Discharged before completion of diagnostic studies | 1 | | 1 | | |
| *Patients without absorption defects* | 8 | 8 | | | |

Hypoparathyroidism 1, cirrhosis of the liver 1.
* Diabetes mellitus.

TABLE 5 *Correlation of duodenojejunal histology and absorption test results*

| Absorption tests | Duodenojejunal histology | | | |
|---|---|---|---|---|
| | Normal mucosa | Slight changes | Partial atrophy | Subtotal trophy |
| Fat + | 6 | 7 | 7 | 1 |
| Fat – | 10 | 2 | 2 | 1 |
| Xylose + | 4 | 4 | 6 | 14 |
| Xylose – | 12 | 5 | 3 | 6 |
| FIGLU + | 2 | 4 | 7 | 17 |
| FIGLU – | 12 | 5 | 0 | 4 |
| Fat + Xylose + | 2 | 2 | 5 | 13 |
| Fat + Xylose – | 4 | 5 | 2 | 6 |
| Fat – Xylose + | 2 | 2 | 1 | 1 |
| Fat – Xylose – | 8 | 0 | 1 | 0 |
| Fat + Xylose + FIGLU + | 0 | 1 | 4 | 10 |
| Fat + Xylose – FIGLU + | 1 | 2 | 2 | 5 |
| Fat – Xylose + FIGLU + | 1 | 1 | 1 | 1 |
| Fat + Xylose + FIGLU – | 1 | 1 | 0 | 3 |
| Fat + Xylose – FIGLU – | 3 | 3 | 0 | 1 |
| Fat – Xylose + FIGLU – | 0 | 1 | 0 | 0 |
| Fat – Xylose – FIGLU – | 8 | 0 | 0 | 0 |

but that normal mucosa was also a possible finding The combined results again give a better correlation. When both faecal fat and xylose excretion or in addition FIGLU were pathological, the mucosa was in most cases severely atrophic There were only two readily understandable exceptionr one patient with resected small bowel and another with sucrose malabsorption.

## Discussion and Conclusions

It is obvious that impaired intestinal absorption is a relatively common finding in children who have both long-standing diarrhoea and failure to thrive However absorption defects may also be detected in patients suffering from diarrhoea or failure to thrive only Whether absorption defects may exist without giving any symptoms at all is not known.

A knowledge of the diagnosti value of absorption tests is of great importance to the clinician. Faecal fat determination has often been recommended as a basic absorption test [13]. Because it measures fat absorption directly this test is subject to few errors as compared with indirect tests. The results of this study support the view [7] that faecal fat is almost al w ys pathological, when there is real disease of the small bowel mucosa or deficiency of fat splitting enzymes. Thus increased faecal fat was a rather unspecific finding which was found in both the primary and secondary malabsorption syndromes and as a transient abnormality per se.

The D-xylose excretion test is specially designed for the detection of malabsorption of upper small bowel origin [6]. Our results are in accordance with this view but a relatively great overlapping of the results in normals and patients is evident in contrast to the results of McCrae [12]

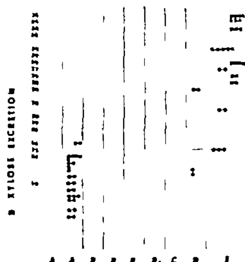


Fig 3. D xylose xcretion in different linical groups. Explanations as in Fig —

parent and when, in addition, the FIOLU test was positive this diagnosis was almost certain Of 44 patients with the primary malabsorption syndrome 4? had at lea t two of these three tests abnormal The pattern of abnormal faecal fat and normal D-xylose FIOLU was typical of pancreatic insufficiency but was also fairly often encountered in other groups especially in malabsorption syndrome associated with systemic diseases

*Duodenojejunal histology*

Table 4 shows that more or less severe histological changes of the small intestinal mucosa were usually observed in patients with absorption defects except in those in whom the defect was due to digestive failure Severe villous atrophy was a typical finding in primary malabsorption.

*Correlation of absorption tests with duodenojejunal histology*

Table 5 shows that abnormal test results pointed to damaged intestinal mucosa

TABLE 4 *Duodenojejunal histology in variou clinical groups*

| Diagnosis | No of patient | Duodenojejunal histology: Normal mucosa | Slight hanges | Partial trophy | Subtotal atrophy |
|---|---|---|---|---|---|
| *Patients with absorption defects* | 48 | 8 | 9 | 9 | 22 |
| Primary malabsorption syndrome | 30 | 1 | | 7 | 20 |
| cow milk intolerance | 10 | | | 3 | 6 |
| gluten intolerance | 20 | 1 | | 4 | 13 |
| Sec malabsorption syndrome | 10 | 6 | 2 | 1 | 1 |
| pancreatic insufficiency | | 2 | | | |
| disaccharide malabsorption | 4 | 3 | | 1 | |
| small bowel resection | 1 | 1 | | | |
| malabsorption syndrome in systemic diseases | 3 | | 2 | | 1[b] |
| Chronic malabsorption syndrome f unknown aetiology | 4 | | 3 | 1 | |
| Transient absorption defect | 3 | 1 | 1 | | 1 |
| Discharged before completion of diagnostic studies | 1 | | 1 | | |
| *Patients without absorption defects* | 8 | 8 | | | |

Hypoparathyroidism 1 cirrhosis of the liver 1
[b] Diabetes mellitus.

## Summary

153 infants and children suspected of having the malabsorption syndrome were studied by means of absorption tests, duodenojejunal histology and clinical follow up. In 88 cases an absorption defect was indicated by faecal fat, D xylose excretion test or disaccharide loadings. The combined symptomatology of abnormal stools and f il re to gain weight appeared to be the best indication of defective absorption. In 57 % of the patients with absorption defects the primary malabsorption syndrome (gluten or cow's milk intolerance) was verified, in 23 % the malabsorption syndrome was secondary and in 20 % non-specific absorption defects were present. Abnormal faecal fat appeared to be the commonest sign of defective absorption, but it was rather unspecific. Abnormal D xylose and FIGLU tests were often found in malabsorption syndrome of intestinal origin. Both of these last-mentioned tests gave some "false positive" and "false negative" results. When the tests were combined, the results were more reliable. The pattern of abnormal faecal fat, D-xylose and FIGLU tests was diagnostic of the primary malabsorption syndrome, whereas increased faecal fat combined with normal results in D-xylose and FIGLU tests was typical of pancreatic insufficiency.


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*Key words*: Malabsorption syndrome, absorption defects, Xylose excretion test, FIGLU test, faecal fat, duodenojejunal histology



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Even when the intestinal mucosal lesion was severe the D-xylose excretion was more than 15 % in many cases (30 of patients with primary malabsorption). Raising the limit to 20 % excretion reduces this number but at the same time increases the number of false positive" results in this series. When 15 has been taken as the limit the number of these false positive results is small. In 10 of normal infants Clark [ ] found D-xylose excretion of less than 15 %. In this series such cases if they exist would be included in the group of transient absorption defects, because in patients belonging to the other groups the malabsorption syndrome was also indicated by other tests and by the histology and clinical picture. Three of the patients in this group had normal faecal fat but abnormal D-xylose excretion. One of these had a pathological mucosa. The remaining two patients may represent "false positive" D-xylose tests. The rate of abnormal D-xylose excretion without other absorption defects is thus apparently lower in our series than in Clark's study namely out of 07 cases. It should be noted that the D-xylose test may give unquestionably normal results in typical primary malabsorption syndrome with gluten intolerance. Also in the patients with diabetes mellitus and malabsorption the D-xylose test gave normal results even in the patient with subtotal villous atrophy. This finding may be due to alterations in xylose metabolism in these patients.

The FIOLU test has not been taken as a basic absorption test in this study because it is known that false positive" results are frequent. According to Frezal *et al* [3], 25 % of non malabsorption patients have positive FIOLU in infancy. In our series 15 % of the patients without other absorption defects had positive FIOLU. In the malabsorption syndrome the FIOLU test follows the same rule as the D-xylose test. An interesting exception was again afforded by the diabetics, who had a positive FIOLU and normal D-xylose test.

Whereas a single test does not give very much information about the final diagnosis and is liable to errors, a combination of tests seems to provide more reliable information. Jones & di Sant Agnese [6] suggested the use of fat absorption studies combined with D-xylose tests for the diagnosis of pancreatic insufficiency. High faecal fat and normal D-xylose excretion was a constant finding in pancreatic insufficiency also in this series. Increased faecal fat with abnormal D-xylose excretion was fairly specific for malabsorption syndrome of intestinal origin. When in addition a FIOLU test was done the specificity was even greater. This is apparently due to the fact that the D-xylose test and the FIOLU test measure the same function i.e. the absorptive capacity of the proximal small bowel. Because each test sometimes gives false positive results greater reliability is achieved by using both.

Our study demonstrates that absorption tests give reliable information on the absorptive function of the small bowel. By a combination of tests it is possible to differentiate between malabsorption of intestinal origin and digestive failure. The final clinical diagnosis can not be made on the basis of the tests discussed here but requires other tests and clinical observation of the patient.

2500 g at birth except one (1935 g). In all cases the Apgar score exceeded 7 at one and ten minutes after delivery. Most of the infants were studied repeatedly at different ages between 30 minutes and 6 days.

## Method

### *pH and blood gas determination*

pH was measured with thermostated Radiometer' standard glass electrode system and a Radiometer PHM 22 at 37°C [7]. All values of blood pH were determined in duplicate. Calculated from 80 consecutive measurements the standard deviation of a single determination was 0.0022.

Standard bicarbonate and in most instances $P_{CO_2}$ were determined according to the microequilibration technique described by Siggaard-Andersen *et al.* [18]. The accuracy of $P_{CO_2}$ and standard bicarbonat measurement by this technique depends mainly upon the accuracy of pH determination, but includes even errors in analyses of $CO_2$-mixtures and for standard bicarbonat errors in buffers. Estimated from the error of pH determination it was 2 per cent for $P_{CO_2}$ and 0,4 mmol/l for standard bicarbonat which corresponds fairly well with the errors found by others [19, 6, 3]. Throughout the study the same buffers were used.

The pH-log $P_{CO_2}$ line being affected by the degree of oxygen saturation, the $P_{CO_2}$ differences obtained by the microequilibration method should be corrected for arterio-capillary oxygen saturation differences. Such a correction could not be performed in 27 paired samples and was therefore omitted for all 61 samples. As the arterio-capillary oxygen saturation differences observed were small (maximum 11%) due to the infants high pH and the relatively high $P_{O_2}$ values in the majority of cases, omission of a correction, however cannot be expected to influence significantly the results. This was shown by testing the 34 paired samples where the arterio-capillary oxygen saturation difference could be determined. When non-corrected $P_{CO_2}$ differences (arterio-capillary mean difference $\bar{D}_1 = -1.99$ mm Hg) were compared with the corrected ones ($\bar{D}_2 = -2.09$ mm Hg) no statistically significant difference was found between the mean differences ($\bar{D} - \bar{D} = 0.1$ mm Hg $p > 0.4$). The maximal difference observed between non-corrected and corrected $P_{CO_2}$ was 1.5 mm Hg

$P_{O_2}$ was determined with a Clark-type electrode [2] and in 34 paired samples $P_{CO_2}$ was measured with a Severinghaus-type electrode [17] at 37°C using the Instrumentation Laboratory Model 113 pH/gas Analyzer. All values are the mean of a duplicate determination. Calculated from 40 consecutive measurements, the standard deviation of a single determination for both $P_{CO_2}$ and $P_{O_2}$ below 100 mm Hg was 0.6 mm Hg

Oxygen saturation was determined spectrophotometrically in the arterial sample or obtained in both the arterial and capillary sample from $P_{O_2}$ and pH by means of a "standard" fetal oxygen dissociation curve [15]. Hemoglobin concentration was determined spectrophotometrically in the arterial sample.

Since the purpose of this study was comparison between arterial and capillary blood no values were corrected for the infants actual body temperature, nor for differences in temperature between arterial and capillary blood.

### *Blood collection and storage*

Arterial and capillary blood sampling was done strictly simultaneously. Arterial blood was obtained from an indwelling umbilical artery catheter (feeding tube French size No. 5). Blood was aspirated into siliconized 5 ml glass syringes, the dead space of which had been filled with a 1 per cent heparin solution. The syringe dead space was taken into account for the final calculation of the result.

Arterialized capillary blood was obtained by a deep (ca 3 mm) heel prick performed some millimeters in front of the distal edge of the calcanean protuberance following a 5–10 minute hyperemisation period in 45–47°C water. Blood usually flowed abundantly without any pressure

being exerted. For pH and $P_{CO_2}$ measurement according to the microequilibration technique blood was sucked into polyethylene tubes (No PE 90) prepared and handled as previously described [7]. For P and direct $P_{CO_2}$ measurement blood was allowed to flow directly into 15 cm long heparinized glass tubes having a capacity of about 0.1 ml. The tubes were immediately sealed with wax and the specimen mixed according to the technique described by Siggaard Andersen *et al.* [18], by using a small piece of wire inserted into the tube and moved by means of a magnet.

Analyses were performed within 40 minutes after sampling and the specimen stored in a refrigerator at 0–4°C until being analyzed. No changes of any significance have been shown to occur during such a lapse of time [7, 8] at the actual gas tensions and storing temperature.

*Statistical methods*

Current statistical methods were used for calculation of standard deviation (S.D.) and for *t*-analyses.

## Results

*pH, carbon dioxide tension and standard bicarbonate*

The infants have been divided into two groups according to their age: Group 1 30 minutes to 24 hours, group 2 48 hours to 6 days. Data concerning pH, $P_{CO_2}$ and standard bicarbonate corresponding to the different age groups have been treated separately.

Comparison between arterial and capillary blood is given in Table 1 and Fig. 1. It can be seen that correlation improves with increasing age. As shown in Table 1 discrepancy is considerable in group 1 with respect to $P_{CO_2}$, capillary $P_{CO_2}$ being on the average 6 mm Hg higher, pH is moderately lower and standard bicarbonate slightly higher in capillary blood. All differences are statistically highly significant. In group 2, there is no statistically significant difference in pH while $P_{CO_2}$ and standard bicarbonate are only very slightly yet statistically significantly higher in capillary blood (0.77 mm Hg and 0.34 mmol/l respectively).

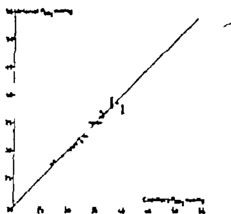


Fig. 1. Arterial $P_{CO_2}$ versus $P_{CO_2}$ in capillary blood sampled from the heel after hyperemization. Filled circles (●) age group 30 minutes to 24 hours, open circles (○) age group 48 hours to 6 days.

The mean arterio-capillary differences for pH, $P_{CO_2}$ and standard bicarbonate in group 1 were significantly different from those obtained in group 2.

There were no statistically significant differences ($p>0.5$, $p>0.1$ and $p>0.05$ for pH, $P_{CO_2}$ and standard bicarbonate differences respectively) between the results obtained in infants delivered by caesarean section ($n=7$) and those born vaginally ($n=22$) in group 1.

*Oxygen tension*

Comparison between arterial and capillary blood is given in Table 2 and Fig. 2. As a correlation to age is not obvious, all data are collected in one group. The dis-

TABLE 1 *Mean difference ($\bar{D}$) between arterial and capillary blood sampled from the heel after hyperemisation*

| | | pH | $P_{CO_2}$ mm Hg | Standard bicarbonat mmol/l |
|---|---|---|---|---|
| Group 1 | n | 24 | 43 | 24 |
| | $\bar{X}$ | 7.337 | 32.2 | 19.0 |
| | | 7.191–7.472 | 22.8–46.8 | 16.0–22.0 |
| | $\bar{D}_1$ | +0.056 | −6.20 | −0.87 |
| | S.D | 0.025 | 5.0 | 1.3 |
| | $P$ | <0.001 | <0.001 | <0.001 |
| Group 2 | n | 42 | 50 | 40 |
| | $\bar{X}$ | 7.348 | 33.3 | 20.6 |
| | | 7.237–7.460 | 24.5–45.0 | 18.0–23.0 |
| | $\bar{D}$ | +0.002 | −0.77 | −0.24 |
| | S.D. | 0.022 | 2.2 | 0.97 |
| | $P$ | <0.25 | <0.01 | <0.05 |
| | $\bar{D}_1-\bar{D}_2$ | 0.054 | −5.43 | −0.63 |
| | $P$ | <0.001 | <0.001 | <0.05 |

Group 1 range of age 30 min to 24 hours.
Group 2: range of age 48 hours to 6 days.
n, number of differences; $\bar{X}$, mean arterial value and range; S.D., standard deviation of the differences; $P$ probability

crepancy between arterial and capillary blood is considerable, $P_{O_2}$ being on the average 9.5 mm Hg lower in capillary blood with a wide range of deviation This difference is statistically highly significant.

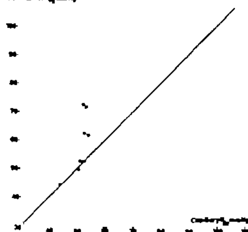


Fig. 2. Arterial $P_{O_2}$ versus $P_{O_2}$ in capillary blood sampled from the heel after hyperemisation. Filled circles (●) age group 5 hours to 24 hours, open circles (○) age group 48 hours to 6 days.

## Discussion

### pH, $P_{CO_2}$ and standard bicarbonate in capillary and arterial blood compared

TABLE 2. *Mean difference ($\bar{D}$) between arterial and capillary blood sampled from the heel after hyperemisation*

Range of age: 5 hours to 6 days.

| | $P_{O_2}$ mm Hg |
|---|---|
| n | 41 |
| $\bar{X}$ | 42.9 |
| | 30.0–63.9 |
| $\bar{D}$ | 9.5 |
| S.D. | 10.0 |
| $P$ | <0.001 |

favourably in infants more than 48 hours of age while discrepancy was considerable as to $P_{CO_2}$ in the age group below 24 hours The difference between the two groups was statistically highly significant (Table 2) With respect to $P_{O_2}$ a large difference was found throughout the neonatal period

The disparity in $P_{CO_2}$ between paired samples in the younger infants is probably due to impairment of the peripheral circulation in the newly born infant An important cause of this impairment is probably vasoconstriction and poor perfusion of the extremities due to low systemic output during the initial circulatory adjustment Left-to-right shunting probably through a patent ductus arteriosus reducing systemic flow may occur until the age of about 15 hours [13] Vasoconstriction in response both to the lower temperature of the extra uterine environment and circulating catecholamines has also been proposed as a possible cause [4]

A similarly good correlation with almost identical mean differences for pH and $P_{CO_2}$ as in the older infants has been obtained by Gandy *et al* [4] in healthy infants after already 3 hours of life A possible explanation may be the fact that in Gandy's study samples were taken between five minutes of each other and not strictly simultaneously which may have contributed towards lowering the arterio-capillary differences provided the arterial sample was taken first Pricking the heel usually elicits violent crying followed by rapid changes in $P_{CO_2}$ and pH However the discordance could also signify that adjustment of the peripheral circulation was achieved more slowly in our material which could be due to those infants that were delivered by caesarean section. Placental transfusion as a determinant factor of blood volume [10] plays a role in the circulatory adjustment [14] It may be impeded by caesarean section unless special techniques are used [16] and cardiac output has indeed been shown to be lower in infants delivered by section rather than vaginally [5] However no statistically significant difference was found between the infants born vaginally and those delivered by section

Discrepancies observed between arterial and capillary $P_{O_2}$ were not unexpected since it is known [9 8] that correlation is unsatisfactory even in healthy adults when capillary blood is sampled from the fingertip rather than the ear lobe Apparently "arterialization" is easier to achieve with respect to $P_{CO_2}$ than to $P_{O_2}$ due to the small arterio-venous $P_{CO_2}$ difference compared to the considerable arterio-venous $P_{O_2}$ gradient No attempt was made in our series to sample from the infants ear lobe For technical reasons simultaneous sampling from an artery arising proximally to the origin of the arterial duct is difficult

At low oxygen tensions the differences between arterial and capillary $P_{O_2}$ tended to be less marked. In some single specimens even higher $P_{O_2}$ values were found in capillary blood. The smaller differences at low tensions can be expected from the shape of the oxygen hemoglobin dissociation curve

It is of course in infants with respiratory and circulatory function impairment that acid base and blood gas studies are of greatest value From experiences in adults [7] however it can be predicted and has actually been shown for $P_{CO_2}$ [4] that

correlation will be still less favourable This emphasizes the need for reliable arterial samples.

## Summary and Conclusion

Arterial and capillary blood obtained by heel prick after previous hyperemisation were compared with respect to pH $P_{CO_2}$, standard bicarbonate and $P_{O_2}$ in infants without major signs of cardiopulmonary dysfunction at different ages during the neonatal period.

Poor correlation was found for pH and $P_{CO_2}$ in the age-group 30 minutes to 24 hours and for $P_{O_2}$ during the entire neonatal period. Over 48 hours of age capillary samples reflected closely the pH and $P_{CO_2}$ of arterial blood For standard bicarbonate a fairly good concordance was found even in the lower age group.

It is concluded that the capillary method using heel blood is rather inappropriate for determination of pH and $P_{CO_2}$ in infants during the first day of life and for measurement of $P_{O_2}$ during the entire neonatal period. It allows only limited conclusions and is subject to large errors in individual cases. It can, however be considered a reliable substitute for arterial blood for pH and $P_{CO_2}$ measurement in infants over two days of age and fairly appropriat for the determination of standard bicarbonate even in the youngest infants.

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# Vitamin $B_6$ Metabolism in Pyridoxine Dependency with Seizures

by J GENTZ, A. HAMFELT S JOHANSSON S LINDSTEDT B PERSSON and R. ZETTERSTRÖM

Familially occurring convulsions in infants, appearing at or shortly after birth and responding promptly to treatment with vitamin $B_6$ in amounts far in excess of the normal requirement have repeatedly been described [5 6 7 9 18 21 22 27]. Since the first report by Hunt, Stokes McCrory & Stouts in 1954 [14] the biochemical mechanism underlying this syndrome has not been established, but it has been suggested that these children have a disturbance in their ability to retain the vitamin in the body [22] The present study reports observations on the metabolism of vitamin $B_6$ in a case of $B_6$ dependent convulsions.

## Case Report

The patient 3-month-old boy was admitted to the Kronprinsessan Lovisas Children Hospital with a history of convulsions commencing t 3 weeks of age The infant was the youngest of six children of healthy unrelated parents. He was born after an uncomplicated pregnancy three weeks before term, with a birth weight of 3130 g The first three weeks of life were normal, but then the mother observed 5 t 10 generalized tonic convulsions daily which were associated with loss of consciousness and lasted for 2 to 3 minutes. H wa breastfed for 5 weeks and thereafter received whole cow milk formula with added saccharose

*Family history* A sister t the patient father has suffered from epilepsia f the Jackson type since the age of 28. A complet neurological examination, including pneumoencephalography carotid angiography and EEG was normal. Her epilepsia is well controlled with antiepileptic drugs. Four f the patient s brothers and sisters ha normal medical histories, but the second child in the family has suffered from epilepsy since 15 months of age. That boy is now 10 years old and mentally retarded. Despit therapy with barbiturates and hydantoin derivates he has generalized convulsions with loss of consciousness two t three times every month. Examination at this hospital t the age of $8_{1/2}$ years revealed a physically normal boy with severe mental retardation and extreme motor restlessness. On the cheeks he had an exanthem consisting of small reddish papules and on the trunk several depigmented spots. N neurological abnormalities were found in the physical examination, but the EEG showed diffuse changes, especially from the left hemisphere A radiogram of the skull showed a small calcification in the region f the pineal body Routine analyses f blood and urine were normal. The urinary

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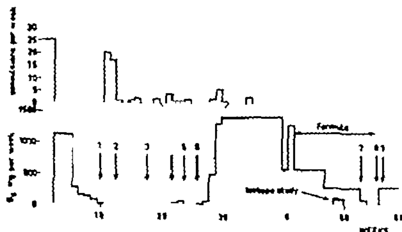



Fig. 1. The effect of therapy with pyridoxine on the frequency of convulsions. At times (1) to (8) the analyses recorded in Table 1 were performed. The study with labeled pyridoxine was carried out during weeks 49 to 50 and the patient received formula diet during weeks 41 to 65.

amino acid pattern was normal. A tryptophan load test resulted in a normal excretion of xanthurenic acid in the urine. The level of pyridoxal phosphate in plasma was also normal. He was started on a supplementary therapy of 40 mg of pyridoxine daily. After 14 months of B-therapy the frequency of convulsion was unchanged, and his mental status remained the same as did the EEG pattern but he seemed more emotionally stable. The exanthem of the face had progressed and microscopic examination of a biopsy specimen showed changes characteristic of sebaceous adenoma. Pneumoencephalography showed a slight atrophy of the right frontal region. Urography was normal.

*Physical examination* on admission revealed a rather plump 3-month-old boy with a weight of 6,480 g and a length of 62 cm. He had a slightly increased muscular tone and was inert. He had repeated generalized tonic convulsions of short duration. Otherwise no definitely abnormal features were noted.

*Laboratory findings.* Hemoglobin concentration was 10.1 g per 100 ml. The white blood cell count and differential count were normal. Normal values were recorded for the serum concentration of sodium, potassium, chloride, calcium, phosphorous, glutamic-oxaloacetic acid (GOT) and glutamic pyruvic acid (GPT) transaminase activities. On admission the alkaline phosphatase activity was elevated (140 to 190 Buch Buch units) but returned to normal values (22, 12 and 14 Buch Buch units) during the following 3 months. The blood glucose level was found to be normal in the fasting state and during period of convulsion. Glucose and insulin tolerance tests were normal. The cerebrospinal fluid contained a normal amount of cells and had a protein content of 63 mg per 100 ml. The urine did not contain any protein, glucose or cells but showed a slight generalized hyper-amino aciduria with a normal pattern on two-dimensional paper chromatography.

*Clinical course.* During a 13-month stay in the hospital the patient received varying amounts of supplementary pyridoxine as shown in Fig. 1. The average number of convulsions per week is also recorded in the same figure. When pyridoxine therapy was instituted during the 2nd week of hospitalization, the convulsions immediately disappeared, the muscular tone became normal and the child became more lively. Withdrawal of pyridoxine on the 10th week resulted in recurrence of the seizures. When pyridoxine was reinstituted during the 20th week no immediate improvement was seen. During the 6th week as many as 25 convul-

sive episodes were observed in one day but after increase of the dose f pyridoxine the clinical condition again improved and the convulsions ceased. The EEG was recorded on 22 occasions. During weeks 1 to 19 the EEG showed a progressive abnormality characterized by sporadic spikes over both hemispheres. In the 20th week the EEG showed a diffuse generalized bnormality with continuous spikes and sharp waves ver both hemispheres. After the 20th week a marked regress in the EEG abnormality was noted, but during the 28th and 29th week the EEG pattern again deteriorated nd now showed a diffuse dysrhythmia with slow waves and sporadic spikes from different parts or occurring in bursts. From the 33rd week the EEG again improved and from the 45th week was considered to be normal. The patient was discharged on a daily supplement f 40 mg of pyridoxine Two weeks after discharge he wa readmitted with high fever and a history of repeated, severe convulsion during the last 24 hours. Pyridoxine administration had no effect on the seizures, but they could be controlled by anticonvulsant drugs. The further course disclosed measles complicated by encephalitis as the cause of this acut relapse. During the following 2 months the patient was free of symptoms except for a slight left sided hemiparesis. The EEG was normal. H was finally discharged at the age f 19 months with daily supplement f 80 mg of pyridoxine

## Special Studies

### *Methods*

*Diet* During the st y in the hospital the patient received a standard infant diet except for the period before and during the isotope study when formula diet of the following composition wa administered. protein (dried skim milk) 16.9 of total calories, fat (soy bean oil) 28.2 of calories, carbohydrates (lactose) 54.9 of calories. A, D and B vitamins were given daily During special diet pyridoxine was administered in dose of 40 mg per day

*Administration of isotope and collection of samples.* Pyridoxine-T H with a specific radioactivity of about 1 μC per μg was obtained from the Radiochemical Center Amersham, Bucks., Great Britain. It gave one single peak on ion-exchange chromatography on a column of Dowex 50. The patient received one intravenous injection of 50 μg of pyridoxine dissolved in 5 ml of physiological saline Urine was collected in 4-hour portions. The storage bottles contained formic acid as preservative and were kept frozen until the time of analysis. Blood samples were collected in heparinized tubes at 1 2, 3, 8, 12 and 31 hours after the administration of the tracer

*Isotope determinations.* The isotope content of the urine and plasma samples and of chromatographic fractions was determined in a Tri Carb liquid scintillation spectrometer (Packard Instrument Co Inc., La Grange Ill U.S.A.) The counting mixture contained 9 ml of methyl cellosolve 10 ml of a toluene solution of 2.5-diphenyl-oxazol (10 g per l) and 1 4-bis 2 (4-methyl-5-phenyloxazolyl)-benzene (0.2 g per l) and 0 4 ml f the urine or plasma samples. The results were corrected for quenching by the addition f 0 1 ml of an aqueous solution of tritium-labeled pyridoxine as an internal standard.

*Determination f urinary excretion f 4 pyridoxic acid* 4-Pyridoxic acid was isolated from 10 ml samples of urine by chromatography on columns of Dowe 2 X8 (200 to 400 mesh, formate form) which were eluted with 0.5 M formic acid in 2 ml fractions. The fluorescence in the effluent was measured at 425 m (activating wavelength 323 nm) in an Aminco-Bowman spectrophotofluorometer (American Instrument Co Sil er Spring, Md. U.S.A.) The fractions containing 4-pyridoxic acid were combined and the isotope content determined. The quant tative determination of 4-pyridoxic acid wa performed fter conversion t the lactone according t the principle of H ff & Perlzweig [13] with the modifications described earlier [15]

*Tryptophane load tests* Xanthurenic acid was determined in the urine according to

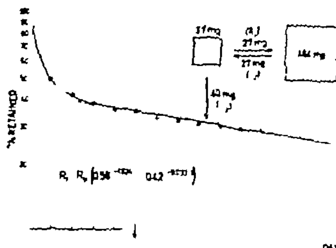


Fig. — Isotope-retention curve after the administration of tritium-labeled pyridoxine. The per cent of administered amount retained in the body at different times ($R_t$) was calculated by subtraction of the cumulative urinary excretion of isotope from the administered amount ($R_0$ = 100). The equation for the curve is given in the figure and the insert shows the previously proposed metabolic model of pyridoxine metabolism with rate constants [illegible] to $k_{[illegible]}$, compartment sizes and transferred amounts.

W [illegible] & [illegible]baum [27] after the oral administration of 0.10 g of L-tryptophane per kg of body weight.

*Determination of pyridoxal phosphate.* The concentration of pyridoxal phosphate was determined in plasma and white blood cells with a tyrosine-decarboxylase method [ ] as described by Hamfelt [10].

*Determination of glutamic-oxaloacetic acid (GOT) and glutamic pyruvic acid transaminase (GPT) activities.* These enzymes were determined in erythrocytes and leucocytes according to Karmen *et al.* [17] as described previously [10].

*Metabolic model for pyridoxine metabolism.* A metabolic model for the overall metabolism of pyridoxine has been described in detail in previous studies [15, 16]. In summary, the amount of isotope remaining in the body at different times after the administration of the isotope ($R_t$) has been shown to follow an equation of the form

$$R_t = R_0 (M [illegible])$$

This equation has been related to an open two-compartment system, consisting of a storage compartment with a slow fractional turnover rate and a compartment with a considerably faster fractional turnover rate as illustrated in Fig. 2. The rate constants for the transfer between the compartments and for the excretion ($k$, $k$ and $k$ in Fig. ) may be calculated from the above equation. A plot of the specific activity of the urinary 4-pyridoxic acid ($q_{A}$) follows the equation

$$q_{A} = q_{A}\left(\frac{\gamma}{\gamma} \cdot \frac{k}{\gamma} e^{\ldots} + \frac{\gamma - k}{\gamma - \gamma} e^{\ldots}\right)$$

from which the amount of vitamin in the smaller compartment may be calculated by the principle of isotope dilution assuming that the urinary 4-pyridoxic acid is derived from this compartment. If the daily intake of vitamin is known, and the subject assumed to be in a steady state this information may be used to calculate the amounts in the two compartments.

## Results

Fig. shows a plot of the amount of isotope retained in the body at different time after the administration of labeled pyridoxine. This curve has been resolved



Fig 2. Radioactivity in blood plasma after the intravenous administration of tritium labeled pyridoxine t the patient.

into two components by subtraction of the second linear component from the first part of the curve and the resulting equa tion for the retention curve is given in the figure Inserted is also a picture of a proposed open two-compartment system for the overall metabolism of pyridoxine, which would result in an isotope-retention curve of the type obtained in this study The calculated compartment sizes, the transfer rates and the excretion rate are included in the figure The slope of the second component of the isotope-retention curve ($\gamma_2$) is 0.033 equal to a half life of 21 days, whereas the first component ($\gamma_1$) had a half life of 0 68 days. Since the slope of the first component of the curve is much larger than the slope of the second component, one may calculate the frac tional loss from the body reservoir of vitamin B as $\gamma_2$, i.e 3.3 per cent per day

Fig 3 shows the isotope content per ml of blood plasma during the first 31 hours following the injection. A rapid fall in the isotope content of the plasma during the first 5 hours after the injection was followed by a slow decline with pproximately the same half life as could be cal culated from the retention curve of total isotope in the body i.e 1 days.

TABLE 1 *Excretion of 4 pyridoxic acid on basic hospital diet and after supplementa tion with pyridoxine in 3 normal subjects (P B H K and M E) and in the patient with pyridoxine-dependent seiz res (P S) and his brother (H S)*

The subjects were given the supplement of pyridoxine for at least 5 days before urine was collected for analysis.

| Subject | Basic (-B) diet + mg pyridoxine per day | Mg 4-pyridoxic acid per day | Addition al pyridoxine excreted as 4 pyridoxic acid ‰ |
|---|---|---|---|
| Normal (P B.) | B | 0.39 | |
| | B | 0.35 | |
| | B + 20 | 10 40 | 5_0 |
| | B + 20 | 10.90 | 53.0 |
| Normal (H. K) | B | 0.56 | |
| | B | 0.63 | |
| | B + 20 | 7 60 | 35.0 |
| | B + "0 | 6.60 | 33.0 |
| Normal (M. E.) | B | 0 61 | |
| | B | 0.40 | |
| | B + 20 | 10 60 | 53.0 |
| | B + 20 | 22.40 | 112.0 |
| Patient (P S) | B | 0.17 | |
| | B | 0 14 | |
| | B + 10 | 2 20 | 22.0 |
| | B + 10 | 1.20 | 12.0 |
| | B + 10 | 1.90 | 19.0 |
| | B + 20 | 2.90 | 14.5 |
| | B + 20 | 2.60 | 13.0 |
| | B + 100 | 17.00 | 17.0 |
| | B + 100 | 21.50 | 21.5 |
| | B + 200 | 4_70 | 21 4 |
| | B + 200 | 31.20 | 15.6 |
| Patient's brother (H. S.) | B + 40 | 22.8 | 57.0 |
| | B + 40 | 28.8 | 72.0 |
| | B + 100 | 48.0 | 48.0 |
| | B + 100 | 49.6 | 49 6 |
| | B + 100 | 34.7 | 34.7 |
| | B + 100 | 47.6 | 47.6 |
| | B + 200 | 90 1 | 45.1 |
| | B + 200 | 109.3 | 54.7 |
| | B + 200 | 91.9 | 46 0 |
| | B + 200 | 107 0 | 53 5 |

The excretion of 4-pyridoxic acid in the urine was determined with a fluorometric method in the patient and his brother and

TABLE 2. *Determinati ns of pyridoxal-5-phosphate in plasma erythrocytes and leucocytes and of GOT and GPT in erythrocytes and leucocytes without* (a) *and with* (b) *addition of pyridoxal 5-phosphat in the assay*

| Analysis | Sampling occasions s indicated in Fig. 1 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Pyridoxal p hosphat in plasma ng/ml | 75.0 | 14 1 | .6.0 | 49.5 | 100 0 | 1.3 | 85.1 | 14 1 |
| Pyridoxal phosphate in erythrocytes ng/erythrocyte | — | — | 0 64 $10^{-}$ | — | — | 1 05 $10^{-}$ | — | — |
| Pyridoxal phosphat i leucocytes ng/leucocyte | — | — | 150 $10^{-}$ | — | — | 146 $10^{-}$ | — | — |
| GOT in erythrocytes | — | — | 4.3 $10^{-}$ ( ) | — | — | 7 6 $10^{-}$ ( ) | — | — |
| µunits/erythrocyte | — | — | 4.6 $10^{-}$ (b) | — | — | 7 7 $10^{-}$ (b) | — | — |
| GOT in leucocytes | — | — | 80 $10^{-}$ ( ) | — | — | 17 $10^{-}$ ( ) | — | — |
| µunits/erythrocyte | — | — | 89 $10^{-}$ (b) | — | — | 17 $10^{-}$ (b) | — | — |
| GPT in erythrocytes | — | — | 0.4 $10^{-}$ ( ) | — | — | 0 7 $10^{-}$ ( ) | — | — |
| µunit /erythrocyt | — | — | 0.4 $10^{-}$ (b) | — | — | 0 6 $10^{-}$ (b) | — | — |
| GPT in leucocytes | — | — | 3.1 $10^{-}$ ( ) | — | — | 3_ $10^{-}$ (a) | — | — |
| µunit /erythrocyte | — | — | 3.9 $10^{-}$ (b) | — | — | 3.8 $10^{-}$ (b) | — | — |

in three normal children aged 10 to 1 years. The excretion was determined when the subjects received the basic diet and after the oral intake of additional pyri doxine The results are given in Table 1 from which it may be seen that the con trols and the patient s brother excreted about 50 per cent of an oral intake of 20 mg of pyridoxine as 4 pyridoxic acid in the urine whereas the corresponding figure for the patient was around 15 per cent The excretion of 4 pyridoxic acid was also determined in the patient after supple mentation of the diet with 10 100 and 200 mg of pyridoxine per day During these periods the excretion of 4-pyridoxic acid ranged from 15 to 20 per cent of the additional intake After the administra tion of the labeled pyridoxine it was pos- sible to calculate the amount of isotope in the 4-pyridoxic acid in per cent of the total amount of isotope in the urine The figure obtained 21 per cent agrees with that obtained by chemical determinations.

The content of pyridoxal phosphate was determined on 8 occasions in the serum and on 2 occasions in the white and red blood cells. The sampling occasions are indicated in Fig 1 The activity of GOT and GPT in the red and white blood cells wa determined with and without the ad dition of pyridoxal phosphate to the assay medium. The results of these studies are given in Table 2 The level of pyridoxal phosphate in plasma when no additional pyridoxine was administered (sampling oc casions 3 6 8) was 14 1 to 26.0 ng per ml and thus fell within the normal range [11] After administration of pyridoxine the concentration increased to between 49.5 and 100 0 ng per ml (see Fig 1 and Table 2) The concentration of pyridoxal phosphate in red and white blood cells (sampling occasions 3 6) also fell within the range of previously reported normal values [1 ] The activities of the trans- aminases in the red and white blood cells determined on the same occasions were

found to be normal and did not increase with the additon of pyridoxal phosphate to the assay medium.

## Discussion

Two types of convulsions have been described in infants related to vitamin $B_6$.

(a) A simple deficiency syndrome due to insufficient intake of the vitamin. These subjects exhibit other signs of depressed activity in vitamin $B_6$-dependent metabolic reactions, e.g an increased excretion of xanthurenic acid in the tryptophan load test [1 2, 3 4 19 20 24]

(b) A familially occurring $B_6$-dependency which requires large amounts of pyridoxine to suppress the convulsions and in which the tryptophan load test has been reported to be normal [5 6 7 9 14, 18 21 22 26]. In this type the fundamental biochemical defect is unknown. Possibly this group may be heterogenous and the clinical manifestations of the disease may vary in different subjects and also be related to the age of the patients.

Although no seizures were observed in our patient until an age of 3 weeks, the criteria for the classification as a case of $B_6$-dependency syndrome are fulfilled. He was admitted at an age of 10 weeks, that is 7 weeks after the start of symptoms. The convulsions disappeared when treatment with 40 mg of pyridoxine per day was instituted, but the EEG pattern did not show a definite improvement until pyridoxine had been given in large doses over a long period of time When pyridoxine wa removed from the diet the convulsions reappeared and the EEG pattern became markedly abnormal once again. Supplementation with up to 500 mg of pyridoxine per week did not cause an immediate improvement and the convulsions did not disappear definitely until the child had received 1400 mg per week for several weeks. Thereafter the supplementation could be reduced to 280 mg per week. A second withdrawal period of 10 days duration did not result in any convulsions, but there was a clinical regression and the child became less alert. Although there were no EEG changes at this time pyridoxine therapy was again instituted. The excessive amounts of vitamin $B_6$ that had been administrated during the previous 25 weeks and the absence of an abnormal loss of vitamin B may have protected the patient from developing more severe neurological symptoms. Tryptophan load tests which were performed before treatment and during one of the withdrawal periods were found to be normal.

Since the $B_6$-dependency syndrome is reported to be familially occurring [26] the occurrence of convulsions since the age of 15 months in one of the patient s 5 siblings and in the sister of the patient s father was of particular interest The brother was examined in detail on two occasions in the hospital and although a definit diagnosis was not obtained, he was apparently not suffering from a $B_6$ dependency syndrome The findings of adenoma sebaceum is suggestive of tuberous sclerosis. There was, however no definite confirmation of this diagnosis from pneumoenceph lographic examination. It is also of interest that he did not show the same pattern of 4-pyridoxic acid excretion a the patient (see Table 1)

In 2 cases of the $B_6$-dependency syndrome an abnormal pattern of excretion

of pyridoxine and its main metabolic end product 4 pyridoxic acid has been reported [22]. In these cases less than usual of the elimination of vitamin $B_6$ was accounted for by 4 pyridoxic acid. In a later report no difference was found [23]. In view of this, the excretion of 4 pyridoxic acid was determined at several levels of daily intake of pyridoxine and control experiments were performed in 3 children. In the normal children from 35 to 55 per cent of the daily intake was excreted as 4 pyridoxic acid which agrees with previous observations in adults [16]. In contrast the patient excreted only about 20 per cent of the daily intake as 4 pyridoxic acid. A low conversion to 4 pyridoxic acid has also been observed in rats suffering from dietary deficiency of vitamin $B_6$ [15].

An excessive loss of vitamin B from the body has been suggested as a possible explanation for the dependency syndrome [3]. In recent experiments with tritium labeled pyridoxine in human subjects [16] it has been demonstrated that a considerable part of an oral dose of pyridoxine is retained in the body and excreted at a very slow rate and the daily elimination from the body reservoir has been estimated to vary from 2 to 3 per cent. The result of the isotope experiment in the present case is not different from the results obtained previously in adults, since the elimination rate from the body reservoir was 2.3 per cent. In view of this it appears that the mechanism of the dependency cannot be an inability to retain vitamin $B_6$ in the body which might be expected from the reported rapid appearance of symptoms after the cessation of therapy with $B_6$. An approximate estimate of the amount of $B_6$ in the body was obtained from the isotope experiment. The value obtained for the total vitamin $B_6$ was considerably higher than noted in previous experiments [16]. No comparison with children of the same age is available and it should furthermore be noted that this study was performed when the patient had received up to 1300 mg per week for 15 weeks. Although the figure is only approximate it does not suggest a diminished amount of $B_6$ in the body. On different occasions the concentration of pyridoxal phosphate was determined in the serum but an abnormally low concentration was never encountered and the administration of additional pyridoxine resulted in increased values for the blood concentration of pyridoxal phosphate. The concentration of pyridoxal phosphate in red blood cell and leucocytes which was determined on two occasions also fell within accepted normal limits indicating that in these cells at least there was no abnormality in the uptake of the vitamin, which is also shown by the fact that the activity of GOT and GPT in the blood cells was not stimulated by the addition of pyridoxal phosphate to the assay medium.

In summary then, we have not found any evidence for an abnormally high rate of elimination of pyridoxine from the body nor any indication of a defect in the penetration of vitamin $B_6$ into the blood cells. The child, however apparently differed from normals in the conversion of pyridoxine to 4 pyridoxic acid. The cause of pyridoxine dependency remains unknown, but possibly structural abnormalities in one or several apoenzymes resulting in increased needs for the coenzyme could

be the underlying cause. Experimental evidence that this type of defect may be the cause of cystathioninuria has recently been presented by Frimpter [8].

## Summary

A 3-month-old boy is described, who suffered from convulsions which disappeared on treatment with pyridoxine in large amounts. After intravenous injection of tritium labeled pyridoxine the elimination of isotope in the urine was followed during 15 days. On the basis of a kinetic model of the metabolism of pyridoxine, the fractional rate of elimination from the body reservoir was calculated as 3.3 per cent per day i.e. of similar magnitude as in normal human subjects. The level of pyridoxal-5-phosphate in plasma and blood cells fell within the normal range and increased after oral administration of pyridoxine. The activity of GOT and GPT in the blood cells was normal and did not increase further after addition of pyridoxal-5-phosphate to the assay medium. The excretion of 4-pyridoxic acid in the urine accounted for 17 to 22 per cent of an oral dose of 10 to 200 mg of pyridoxine in the patient as compared to about 50 per cent in 3 normal subjects and in the patient's brother.

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# Correlations between Ultrasonic and Roentgenologic Findings in Infantile Hydrocephalus

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The ventricular system of the brain can be visualized by conventional intracranial pneumography The size of the lateral ventricles can also be assessed by cerebral angiography

These methods are still the best for demonstration of the ventricular system, but they carry certain risks and in the examination of infants general anaesthesia is often necessary A simpler method of examination of the ventricular system in infants is therefore desirable

Since 194 when Dussik [7] introduced the use of ultrasound in the examination of the ventricular system of the brain rapid advances have been made in this field. Dussik used the so-called transmission method for charting the ventricular system and measured the weakening of ultrasound on its passage through brain tissue and ventricular fluid. The method has however not been widely used [, 3]

In 1955/56 Leksell [11] used the echo-method for demonstrating post traumatic intracranial complications by estimating the shift of a midline echo This echo derived from some structure normally situated in the midsagittal plane f the skull The discovery was followed by a surge of interest in the diagnostic use of ultrasound, though it was not thought that the echo-technique could ever substitute for pneumoencephalography [9] This view still holds with regard to such causal factors of hydrocephalus as arachnoiditis obstruction of the aqueduct and papillomas of the choroid plexus. So far it has not been possible to demonstrate these conditions by echoencephalography On the other hand echoes can be obtained from the walls of the lateral ventricles and the third ventricle in children [5 6 10 13 15 16 18].

A fairly thorough search of the literature failed to reveal any systematic comparison of the echo-ventriculographic findings with the intracranial pneumographic findings in infants with hydrocephalus. Such a comparison was therefore considered desirable nd the results are given below

## Method of Examination

An ultrasound apparatus (Krautkrämer model USIP 9) and a transducer containing a barium titanat crystal 10 mm in diameter and working w th frequency of 2 MHz were used. The same pparatus was used by Jeppsson [10], who also described its characteristic working properties. Paraffin oil acted as contact substance between the

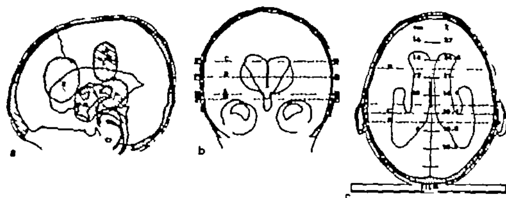


Fig 1 (a) Schematic picture of the entricular system and actual head areas A B C and D used for application of the transducer (b) The intracranial ultrasound beam directions in the frontal projection in case of moderat hydrocephalus, P transducer ( ) Horizontal section through the skull showing directions of ultrasound beam, distances to the X ray film and the X-ray magnifications.

transducer and unbro en skin. The patient was examined either in the sitting or recumbent position. No premedicants were used.

The midline echo was identified in the conventional way by placing the transducer against the skin in th temporal region about 4 cm cranial to the xternal auditory meatus in the vertical plane of the ear (area A, Fig 1 ) and by dir ecting the ultrasound beam at right angles to the cranial midsa ttal plane

The width of the third ventricle was determined by placing transducer about 2 cm ventral to the ext al auditory meatus at the level of the cranial der of the auricle (area B Fig 1a) The d nce in the echoventriculogram, between he two central amplitudes, representing th lateral walls of the third ventricle was re ded and measured.

The horizontal width f lateral ventricles was measured with transducer placed about 6 cm cranial t he external auditory meatus and about 2 m ventral to the vertical plane of the ear (a ea C, Fig 1a) and with the transducer pl aced over the region of the coronal suture a out 3 cm cranial to the zygomatic arch (are D Fig 1a) In this way the horizontal wid th of the lateral ventricles was measured over the body region and over the dorsal part of the anterior horns.

The transducer acted as a transmitter and receiver Records were made with the transducer on the right as well on the left side of the head. However when checking the position of the cranial midline [10 12, 16] one transducer in area A (Fig. 1a) on one side of the head acted as a transmitter and another transducer in the same area on the opposit side of the head acted as receiver

The tracings on the cathode-ray oscillograph were photographed with a Polaroid Land camera, and the photographic records were developed immediately The echo distance between the midline echo amplitude and the echo amplitude corresponding to the lateral wall of the lateral ventricle (L-V echo) as well as the distance between the amplitude of the L-V echo and the echo amplitude corresponding to the inner surface of the skull was measured in millimetres and the value obtained was multiplied by the factor 4 0. This factor was calculated from a comparison of the measurements obtained in 55 children by measuring the distance between the skin areas A (Fig. 1a)

with a pelvimeter and by measuring the same distance using the ultrasound transmission method It was found that 1 mm in the echo-ventriculogram corresponded to 4.0 mm in the head.

The values obtained were regarded a measures of the horizontal width of the third ventricle, and f the horizontal width of the lateral ventricles over the body region and over the dorsal part of the anterior horns. The thickness of the brain parenchyma over the parietal region was also measured (area C, Fig 1 ). Corresponding regions were identified by pneumoencephalography or ventriculography and measured by the author and after correction of the X ray enlargement, compared with the echo- en triculographic values (Figs. 1b and 1 ) The dist nces measured in the X ray films represented the basic values and were com pared with corresponding distances measured in the echo-ventriculograms (Figs. 2a and 2b). The differences, expressed in per cent between the distances measured were regarded as positi e (+) when the distances in the echo- entriculogram were larger than the corresponding distances in the roentgenogram. Otherwise the percentage dif ference was said t be negati (−)

## Material and Results

The material consisted of 30 children (16 girls and 14 boys) aged 1 week to 106 months. Most of the children were below 6 months. The echo-ventriculographic examinations were carried out at most 10 days before or after intracranial pneumography Only distances that corresponded to one another in both X ray films and echo-ventriculograms were accepted. The results are presented in T bles 1 and 3

The widths of the bodies of the lateral ventricles and the thickness of the parietal brain parenchyma were measured echoventriculographically and roentgenologi cally in 22 children. In cases the bodies of the lateral ventricles and the parietal brain parenchyma were not visualized on X ray films because of incomplete filling of the ventricles with air and in 1 case the bodies and the brain parenchyma were not investigated with echo-ventriculography

In 15 cases the right and in 16 cases the left anterior horns of the lateral ventricles could be compared. The anterior horns on the right side in 3 cases and on the left in cases were not clearly outlined in X ray films. These parts were not investigated echo-ventriculographically in 10 cases, in 1 case no midline echo could be identified, and in 1 case th echo-ventri culogram failed to show well defined L-V echoes (Tables 1 and 2)

Only in 11 of these 30 cases were com parisons made between the roentgenogra phic and the echo-ventriculographic width of the third ventricle. In 9 cases the third ventricle could not be identified with th s echo-ventriculographic technique and in 5 cases this part of the ventricular syst m was not investigated with ultrasound. In 5 cases the third ventricle could not be identified in the X ray films (Table 2)

The mean values of the differences in per cent and standard deviations are given in Table 3.

## Discussion

Infantile hydrocephalus can, as rule be readily recognized from historical data and clinical manifestations. Valuable supplementary information on the size of the ventricles and the thickness of the brain parenchyma can be obtained by echoventriculography In the present 30 cases f irly good agreement was found between the echo-ventriculographic and neuro-

TABLE 1 *The width of the lateral ventricles*

R = right L = left

| Case | Age (mo.) | By ultrasound in mm: Body R | Body L | Anterior horn R | Anterior horn L | By X ray in mm: Body R | Body L | Anterior horn R | Anterior horn L | Difference in per cent: Body R | Body L | Anterior horn R | Anterior horn L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L. R. | 1 | 26.0 | 45.1 | 28.7 | 28.7 | 33.6 | 48.7 | 31.2 | 31.2 | + 8.9 | − 7.0 | − 8.7 | − 8.7 |
| L. K. | 1 | — | — | 45.1 | 45.1 | — | — | 24.0 | 24.0 | — | — | +46.9 | +46.8 |
| L. L. O | 1 | 32.8 | 32.8 | — | — | 48.9 | 48.9 | — | — | 18.0 | +18.0 | — | — |
| S. S | 1 | 28.7 | 28.7 | — | — | 35.3 | 35 | — | — | +1[illegible].2 | +1[illegible].2 | — | — |
| J. L. | 1 | 8.7 | 28.7 | — | — | 16.8 | 16.8 | — | — | +41.5 | +41.5 | — | — |
| C. L. | 1 | 28.7 | 28.7 | — | — | 20.1 | 20.1 | — | — | +30.0 | +30.0 | — | — |
| R. Z. | 1 | — | — | 20.5 | 20.5 | — | | 13.1 | 40.0 | — | — | +35.1 | + 4 |
| K. A. H | 2 | 28.7 | 34.0 | 24.6 | 30.8 | 22.7 | 40.4 | 18.5 | 24.6 | +20.0 | +15.8 | + 4.8 | +20.1 |
| L. N | 2 | 65.6 | 24.6 | — | — | 54.5 | 27.7 | — | — | +16.9 | 12.6 | — | — |
| L. R. | 2 | 32.8 | 32.8 | 34.0 | 44.1 | 33.4 | 33.8 | 29.6 | 33.0 | − 2.4 | − 2.4 | +15.2 | + 3.2 |
| N. N | 2 | — | — | — | 24.8 | — | — | — | 6.9 | — | — | — | − 0.3 |
| K. J | 3 | 61.5 | 61.5 | — | — | 50.3 | 50.3 | — | — | +18.2 | +18.2 | — | — |
| M. S | 3 | 41.0 | 41.0 | 28.7 | 28.7 | 21.8 | 1.8 | 21.5 | 21.5 | 46.8 | +46.8 | +[illegible]5.1 | +[illegible]3.1 |
| G. R. | 4 | — | — | 36.9 | 36.9 | — | — | 4[illegible].3 | 47.7 | — | — | −14.6 | +24.9 |
| J. H | 4 | 40.3 | 40.3 | — | — | 45.3 | 45.3 | — | — | + 7.0 | + 7.9 | — | — |
| A. F | 4 | — | — | 19.7 | 19.7 | — | — | 23.1 | 23.1 | — | — | −17.2 | −17.[illegible] |
| M. N | 6 | — | — | 32.8 | 32.8 | — | — | [illegible]2.2 | [illegible]0.[illegible] | — | — | +11.0 | +11.0 |
| Th. J | 6 | 28.7 | 30.8 | 3[illegible].0 | 32.6 | 31.9 | 35.1 | 31.0 | 35.1 | −11.3 | −14.7 | + 0.3 | − 4.6 |
| F. H | 8 | [illegible]8.7 | 28.7 | — | — | 46.0 | 46.0 | — | — | + 9.4 | + 9.4 | — | — |
| R. J | 8 | — | — | 41.0 | 49.3 | — | — | 29.5 | 47.7 | — | — | + 6.1 | + 3.0 |
| K. L. | 10 | 57.4 | 57.4 | 61.5 | 61.5 | 55.4 | 55.4 | 53.8 | 53.8 | + 3.4 | + 3.4 | +1[illegible].5 | +12.5 |
| E. P | 18 | 20.5 | 20.5 | | — | 1.0 | 21.0 | — | — | − 4 | − [illegible]4 | — | — |
| U. H. | 23 | 32.8 | 41.0 | — | — | 35.2 | 35.[illegible] | | — | − 7.3 | +14.1 | — | — |
| M. H | 44 | 61.3 | 51.3 | — | — | 42.0 | 42.0 | — | — | +18.1 | +18.1 | — | — |
| M. K | 44 | 36.9 | 36.0 | 28.7 | 28.7 | 40.4 | 20.4 | 20.4 | 25.4 | +20.3 | +20.3 | +11.5 | +11.5 |
| B. O. J | 40 | — | — | 41.0 | 57.4 | — | — | 34.6 | 74.6 | — | — | +15.6 | −20.0 |
| M. R. | 61 | 24.6 | 24.6 | | — | 23.7 | 25.7 | — | — | − 4.5 | − 4.5 | — | — |
| R. M. R. | 61 | 41.0 | 47.2 | — | | 37.8 | 47.8 | — | — | + 7.8 | − 1.3 | — | — |
| M. J | 0 | 18.5 | 18.6 | 15.5 | 15.6 | 18.5 | 18.8 | 19.2 | 19.2 | 0.0 | 0.0 | −23.1 | −23.1 |
| O. M. P | 108 | 40.1 | 45.1 | | — | 52.1 | 52.1 | | — | 15.5 | −15.5 | — | — |

TABLE 2 *Brain parenchyma and the third ventricle*

R = right. L = left

| Case | Age (mo.) | Thickness of the brain parenchyma parietally in mm and difference in %: By ultrasound R | By ultrasound L | By X-ray R | By X-ray L | Difference in % R | Difference in % L | Width of the third ventricle and diff. in %: By ultra sound | By X-ray | Diff in % |
|---|---|---|---|---|---|---|---|---|---|---|
| L. S. | 1 | 8.2 | 8.2 | 8.4 | 8.4 | − 2.4 | − 2.4 | — | — | — |
| L.-L. G | 1 | 16.4 | 16.4 | 15.9 | 18.5 | + 3.1 | −12.8 | 8.2 | 7.4 | − 9.8 |
| S. S. | 1 | 20.5 | 20.5 | 21.0 | 21.0 | − 2.4 | − 2.4 | 6.2 | 5.8 | + 6.5 |
| J. L. | 1 | 18.5 | 15.5 | 25.2 | 25.2 | −26.2 | −26.2 | 16.4 | 8.3 | −49.4 |
| C. L. | 1 | 16.5 | 16.5 | 16.8 | 15.1 | + 9.2 | +16.4 | 8.2 | 8.2 | 0.0 |
| K. Å. H. | 2 | 16.5 | 18.5 | 1.0 | 27.7 | −13.5 | −49.7 | 8.2 | 9.1 | −11.0 |
| L. K. | 2 | 3.3 | 12.3 | 2.5 | 12.6 | +24.2 | − 2.4 | — | — | — |
| L. E. | 2 | 16.4 | 16.4 | 16.8 | 16.8 | − 2.4 | − 2.4 | — | — | — |
| X. X. | 2 | — | — | — | — | — | — | 12.3 | 9.1 | −26.0 |
| K. J. | 3 | 8.2 | 10.3 | 7.6 | 10.1 | + 7.3 | + 1.9 | 24.6 | 18.2 | +26.0 |
| M. S. | 3 | 24.6 | 22.8 | 34.4 | 26.0 | −28.8 | − 9.– | 8.2 | 9.9 | −20 |
| J. H. | 4 | 2.0 | 2.0 | 1.7 | 1.7 | +15.0 | +15.0 | 14.4 | 10 | −.5 |
| Th. J. | 6 | 25.4 | 25.4 | 23.5 | 23.5 | + 7.5 | + 7.5 | — | — | — |
| F. H. | 8 | 24.6 | 28.7 | 26.9 | 21.0 | − 9.4 | +26.9 | — | — | — |
| R. J. | 8 | — | — | — | — | — | — | 11.5 | 11.6 | − 0.9 |
| K. L. | 10 | 12.3 | 12.3 | 10.9 | 10.9 | +11.4 | +11.4 | 16.4 | 10.0 | −39.0 |
| E. P. | 18 | 53.3 | 45.1 | 45.3 | 42.0 | +15.0 | + 6.4 | — | — | — |
| U. H. | 22 | 18.5 | 18.5 | 22.7 | 22.7 | −22.7 | −22.7 | — | — | — |
| M. H. | 44 | 24.6 | 24.6 | 25.2 | 25.2 | − 2.4 | − 2.4 | — | — | — |
| M. K. | 44 | 24.6 | 24.6 | 29.4 | 29.4 | −19.5 | −19.5 | — | — | — |
| M. E. | 61 | 28.7 | 28.7 | 32.5 | 32.5 | −13.2 | −13.– | — | — | — |
| R. M. R. | 61 | 21.3 | 21.3 | 19.3 | 19.3 | + 9.4 | + 9.4 | — | — | — |
| M. J. | 70 | 45.1 | 45.1 | 46.1 | 46.1 | − 2.2 | − 2.2 | — | — | — |
| G.-M. P. | 106 | 24.4 | 24.4 | 22.7 | 22.7 | + 7.0 | + 7.0 | — | — | — |

roentgenologic findings. Only in 1 case, which was not included in the present material, have we ever found any discrepancy between the echo-ventriculographic and roentgenographic findings when using the technique described above. This case was examined in the beginning of the present series and the misleading result can probably be ascribed to the limited experience of the examiner.

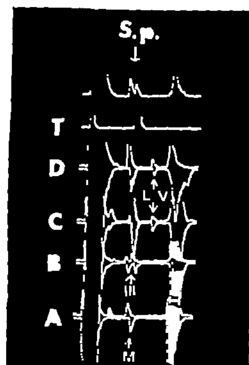
TABLE 3 *Mean values of the differences in per cent and standard deviations*

R = right. L = left

| | Lateral ventricles: Body R (22)* | Body L (22) | Anterior horns R (15) | Anterior horns L (16) | Brain parenchyma R (22) | Brain parenchyma L (22) | Third ventricle (11) |
|---|---|---|---|---|---|---|---|
| Mean | +10.77 | 8.63 | +9.42 | −5.60 | 2.59 | 3.35 | −12.6 |
| S.D. | ±15.9 | ±16.8 | ±16.7 | ±20.7 | ±16.4 | ±17.6 | ±1.5 |

* Number of cases in parentheses.




( ) (b)

Fig. — ( ) Pneumoencephalogram showing moderat hydrocephalus and a cyst f septum pellu cidum (Th. J 6 months old). (*b*) Echo-ventriculographic tracings from the same case as in 2*a*. In this well as in the following recordings the upper tracing in each pair w taken with the transducer on the right le of the head, the lower tracing with the transducer on the left. *A* *B* *C* and *D* correspond t the areas outlined in Fig. 1 *A* displacement f the midline echo by 4 mm t the right The midline echo (*M*) is seen n the middle of the tracings. *B* Two echo mplitudes (III), representing the width of the third entricle are seen in the middle part of tracings. *C* The distance between midline echo and lateral entricle echo (L–V) represent the wi f the bodies of the lateral entricles nd the distance between L–V echo nd bottom echo (l echo mplitude furthest to the right in every echo-ventriculographic tracing) represents the thi ness f the brain parenchyma parietally *D* the width of the anterior horns (dist nce from m line echo t L–V echo) and the thickness of the frontolateral brain parenchyma (distance fr L–V echo t bottom echo) are seen in these tracings. *T* cranial midline control. *S–P* fro lateral recording of two echoes (*arrow*), which seem t emanat from the walls f the septu pellucidum cyst seen in 2*a*.

Judging from experience gained in the present investigation it is easiest to outline the bodies of the lateral ventricles and the brain parenchyma parietally The anterior horns are more difficult to identify probably because of the narrowing of the skull in the frontal direction. The ultrasound beam is thus not perpendicular to the walls of the frontal horns with the result that the sound is not reflected onto t transducer The difficulty in identifyi the third ventricle by ultrasound c probably be explained in the same wa for in hydrocephalic children the later walls of the third ventricle are most ofte oblique

The individual differences, seen i Tables 1 and 2 between the echo-ventr

culographic and X ray values may be explained by the individual variations in the length of the skull and by the shortness of the tracings. The individual variations in the length of the skull not taken into account in this investigation, result in different values of X ray enlargement The shortness of the tracings made exact evaluation of them somewhat difficult.

When outlining the supratentorial ven tricular system with the technique men tioned above the narrow ultrasound beam will not strike the temporal horns because of their topographical position, except when outlining the third ventricle The echo amplitudes which then seem to emanate from the temporal horns are to be seen laterally in the echo-ventriculo-gram and can, with some experience be readily distinguished from the two more centrally situated echo amplitudes from the walls of the third ventricle.

In hydrocephalic children the lateral ventricles seem to dilate fairly symmetri cally and the estimation of the width of the frontal horns serves as a good control of the estimated width of the bodies.

As pointed out by Sjögren [15] the L-V echo in the echo-ventriculogram is charac terized by a sharply outlined echo ampli tude bordering a zone that is echo-free because of the ventricular fluid. At the junction between the brain parenchyma and a subdural effusion in a non hydro-cephalic child a sharp echo amplitude is sometimes to be seen, too but then there is no echo-free zone because of the multi-echo properties of the brain parenchyma. This condition can therefore hardly be mistaken for hydrocephalic changes.

It is seen in Table 3 that the values obtained by outlining the ventricular

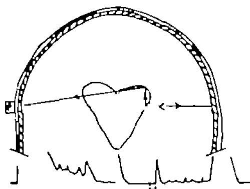


Fig 3. Schematic picture showing a frontal sec tion of the head and the reflexion f ultrasound between adjacent part of the concave en tricular wall resulting in lengthening ($x$) of the distance between midline echo and L-V echo in the echo-ventriculogram.

system are somewhat too great and those obtained by estimating the thickness of the brain parenchyma somewhat too small. As shown in Fig 3 this can at least partly be explained by reflection of ultrasound between adjacent parts of oblique ventricular walls. In the echo-ventriculogram this may result in a length ening of the distance between the mid line echo amplitude and the L-V echo amplitude and a shortening of the distance between the L-V echo amplitude and bottom echo amplitude.

The echo-ventriculographic method has proved simple safe quick, painless and a valuable supplementary procedure in the clinical evaluation of assumed hydroce-phalus. Repeated studies at shorter (Figs. 4a b c) intervals showed the method to be reliable. Proper use of the echo-ventri culographic method requires experience and some knowledge about the physical properties of the ultrasound as well as about the topography of the cerebral structures.

With echo-ventriculography it i also possible to exclude hydrocephalus in infants. In such cases recording in areas C and D (Fig 1a) shows two amplitudes on either side of and close to a midline echo These amplitudes seem to emanate from the walls of the lateral ventricles.

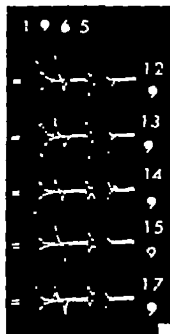



Fig 5 shows that the echo-complex situated in the middle then assumes the appearance of a 3-pointed crown. As pointed out by de Vlieger & Ridder [16] and Lithander [14] the walls of the third ventricle produce a double midline echo amplitude This can be seen (B in Fig. 5) in a patient with a normal ventricular system.

Future methods will probably enable two-dimensional ultrasonic examination of the ventricular system and of other intracranial normal and pathological components. Attempts at such charting of the ventricular system have already been made [1 4 8, 17]. Preliminary examinations have also been made at our department and the results seem promising

Fig. 4 (a) Echo- entriculographic findings over area C (Fig 1) showing advanced enlargement of the lateral ventricles and a thin parietal brain parenchyma in day to day investigations in an infant born 10th Sept 1965.

(b and ) The thin parietal brain parenchyma visible in pneumoencephalogram taken 1 th Sept 1965 from the same case





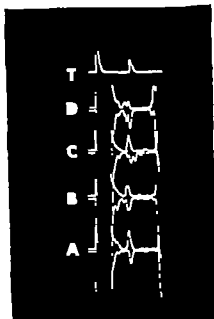



Fig. 5 A normal echo-ventriculogram in a 1-month-old infant showing the midline echo without displacement (A), the double peak-echo corresponding to the third ventricle region (B), the three peak-echo corresponding t the lateral ventricle regions (C D) and the cranial midline control (T).

## Summary

The A-scan technique for ultrasonic demonstration of the supratentorial ven tricular system in infants is described.

30 infants with hydrocephalus verified by intracranial pneumography were exa mined by echo-ventriculography The thickness of the parietal brain parenchyma the widths of the third ventricle of the bodies and of the anterior horns of the lateral ventricles were measured in the X-ray films and in the echo-ventriculograms. The results were compared. Fairly good agreement was found between the results obtained by the two methods.

The echo-ventriculographic findings in infants with a normal cerebral ventricular system are discussed.

It is pointed out that the echo-ventricu lographic method is safe quick, painless and reliable. Proper use of the method requires some experience and also some knowledge of the physics of ultrasound as well as of the cerebral topography

## Acknowledgement

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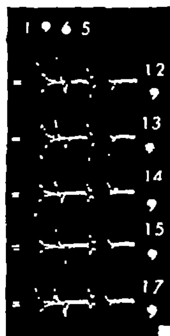


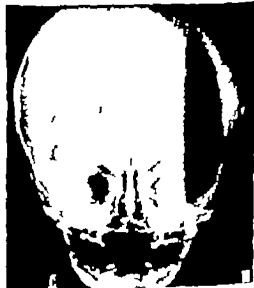

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Future methods will probably enable two-dimensional ultrasonic examination of the ventricular system and of other intracranial normal and pathological components Attempts at such charting of the ventricular system have already been made [1 4 8 17] Preliminary examinations have also been made at our department and the results seem promising

Fig. 4 ( ) Echo-ventriculographic findings over area C (Fig. 1) showing advanced enlargement of the lateral ventricles and a thin parietal brain parenchyma in day t day investigations in an infant born 10th Sept. 1965.

(b and ) The thin parietal brain parenchyma visible in pneumoencephalogram taken 17th Sept 1965 from the same case





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From the Departments of Pediatrics and Chemistry II of Karolinska Institutet Stockholm, Sweden

# The Normal Venous Plasma Free Amino Acid Levels of Non Pregnant Women and of Mother and Child during Delivery

*by* B. S LINDBLAD and A. BALDESTEN

The active transport of amino acids from mother to foetus has been well documented by Christensen's & Streicher's finding of competitive transport in guinea pigs [9] Dancis *et al* discovery of the saturation of the transport mechanism of histidine in guinea pigs [14], and Page's *et al* discovery of stereospecificity in the transplacental transport of histidine in homo [43]. Thus, besides simple diffusion, there seems to exist a selective biochemical system for the transport of amino acids in the placenta which may be compared to the reabsorption in the renal tubuli or the active transport in the intestinal mucosa

A simultaneous study of the complet amino acid pattern in the plasma of mother and foetus could provide valuable information about the foetal protein metabolism and the foetal requirements. Present-day knowledge is based on the results of the analysis, by different methods, of a limited number of samples Clinical correlation is often lacking and there is scant information about the procedures for collection, storage and deproteinization.

The present study aims at providing a basis for future studies of the amino acid levels under clinically well-defined pathological conditions. Consequently emphasis has been laid on clinical correlation and standardized procedure.

## Methods

### *Collection of samples*

At delivery the umbilical vein was carefully cleaned after cessation of the pulsations. The umbilical vein was incised and blood collected in a heparinized tube. All unnecessary handling of the cord was avoided Less than 5 minutes after the collection from the cord, blood was drawn from the mother cubital vein. The samples were centrifuged immediately and the plasma withdrawn. If haemolysis occurred the samples were discarded. The plasma was then frozen at −20°C until it was deproteinized less than 6 hours later

Contamination from the mother blood and the amniotic fluid on the outside of the cord was avoided Isolated collection from the umbilical vein was necessary owing to the known arterio-venous differences [45, 50, 37 44]. The red corpuscles of the newborn

This study was made possible through grant from Expressens Pre-Natal Fund and A/B Semper Fund for Nutritional Research.

child are especially liable to haemolysis and maltreatment of the cord, e.g. in the form of the common "milking" caused haemolysis. Similarly, if the plasma was not separated immediately, haemolysis would occur. Owing to the great concentration of amino acids in the red corpuscles [4, 33, 30] haemolysis must be considered a source of possible gross errors. Plasma stored at −20 C will show loss of cystine (protein binding of cysteine) while aspartic acid and glutamic acid will increase [53]. The speed of these changes has been estimated and the slight loss of methionine upon prolonged storage (8 months) has also been shown [18].

*Deproteinization*

A modification of the general picric acid method of Hamilton & Van Slyke [26] was used. To 1 ml of plasma was added 1 ml of a reference solution (alpha-amino-beta-guanido-propionic-acid: AGP) and 4 ml of saturated picric acid. After thorough mixing the suspension was centrifuged, the supernatant withdrawn and 5 ml chromatographed on a Dowex 1 × 10 ion exchange column, 4 × 50 mm, in the chloride form [53]. Elution was performed with 5 × 1 ml of 0.02 N HCl. The effluent was lyophilized, the dry sample dissolved *in 3 ml of water, pH brought to* 7–8 with sodium hydroxide and the solution left for 4 hours at room temperature to convert cysteine to cystine [53]. The oxidized sample was brought to pH 2 with hydrochloric acid, freeze-dried and stored at −20°C until analysis.

Deproteinization by picric acid was chosen *in accordance with the investigations done* by Stein & Moore [53] who compared equilibrium dialysis, ultra-filtration and picric acid and showed the most favourable results upon using the picric acid method. Oepen [40] was able to show the highest amino acid levels after picric acid deproteinization in a series of differently deproteinized samples. Similar results were reported by Gerritsen *et al.* [22] concerning the sulfosalicylic acid method contra the picric acid method. Small amounts of protein do not interfere with the analysis [27, 23], but in our hands the method of partial removal of protein by ultra-centrifugation [23] clogged the columns. The method was also abandoned because of the impossibility of storage owing to the changes in not deproteinized plasma at −20 C [18].

Excessive picric acid has to be removed as it disturbs the colorimetric reading at 570 mμ giving a peak in between that of taurine and urea [18]. Two samples of picric acid with an addition of Beckman standard solution (containing 17 amino acids) were run through the entire procedure without finding any losses of the amino acids concerned.

*Chromatography*

The sample was dissolved in a pH 2.2 citrate buffer and chromatographed on a Beckman 120 B amino acid analyzer adapted for fast runs and with a high sensitivity cuvette system [1, 2]. This analyzer is a modification of the ion-exchange chromatography of amino acids according to Moore & Stein [33].

Alpha-amino-gamma-guanido-butyric acid (AGB) and n-leucine were used as internal standards. The standard procedure was used without any temperature gradient except *for the separation of the basic amino acids,* where a column with the resin Beckman Type AA 27, 9 × 180 mm and the following citrate buffer system was used: 0–10 minutes the pH 5.28 buffer, 10–25 minutes the pH 4.26 buffer and from 25 minutes the pH 5.28 buffer again. This made it possible to separate ornithine from lysine and it did not affect the separation of the other basic amino acids. The run lasted 160 minutes with a flow rate of 72 ml per hour and temperature of 55°C.

A representative chart from our analyses is shown in Fig. 1 and 2. A stable base line was achieved in all the analyses. All the amino acids reported here could be plotted by the height times-width method alone [52]. A smaller peak was always observed before the one representing aspartic acid in the position of methionine-sulfone and hydroxyproline. In our system the peaks for threo-



Fig 1 Chart no 421 a. Chromatogram showing the acidic and neutral amino acids plus urea in the plasma of a normal pregnant woman during delivery Nor-leucine was add d t the sample as internal standard for colour yield.

nine and serine were overlapped by the peaks for glutamine and asparagine. In order to form an estimation of their indicated area, 3–4 samples in each group were analyzed before and after acid hydrolysis (HCl + 100°C for 2 hours) of the deproteinized plasma [57 53]. Glutamine and asparagine were thereby converted to glutamic and aspartic acid and their approximate levels could be estimated from the increase in glutamic and aspartic acid received. The peaks of threonine and serine were well separated after hydrolysis. The results of this survey are separated from those of the unhydrolized samples in the presentation below

Cystine has proved to be an especially difficult amino acid t reproduce by this method. Butterfield & O'Brien [7], put its reproducibility t ±10% compared t ±6 in the other amino acids. This is partly because cystine in its natural form, cysteine has tendency t disappear through handling and storage of the sample and partly because of the high sensitivity to slight hanges in buffer pH which easily gives verlapping with the peak representing alpha-amino-butyric acid. A comparison of the range of the individual amino acid determinations in our material of non pregnant women with the adult levels in the literature as reviewed by Soupart [51], shows lower values only for cystine (see Fig 3). Cystine has therefore been separated in the present tion of the results below

The peak representing the internal stan dard of leucine comes directly after that of leucine and is invariably well separated from t In the samples from the mothers the ornithine-peak was sometimes partly overlapped by that of lysine and not plotted. In the first six pairs of samples analyzed, carnosine was added to the plasma as an internal standard. W observed however a loss of carnosine and an increase of the histidine-peak, pparently because of plasma dipeptidase-activity (carnosine being bet alanyl-l-histidine) [t be published]. Consequentl carnosine was abandoned as an internal standard in f vour of AGP and the 6 determinations with the incorrect histidine values were discarded A small unidentified

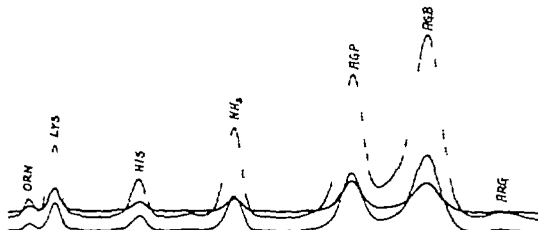



Fig. — Chart no. 4216. Chromatogram showing the basic amino acids in the plasma of normal pregnant woman during delivery. AGP was added to the undeproteinized plasma and AGB was added to the deproteinized plasma as internal standards.

peak was seen between histidine and ammonia.

Tryptophane was not traced in our analyses. Tryptophane is partly free, partly bound to the plasma-albumine fraction [42] and a certain difference in the analyses of not deproteinized contra deproteinized plasma is to be expected with regard to this amino acid. Tryptophane is also lost upon depierinization using Dowex according to Stein & Moore [63]. The internal standard of AGP and AGB are represented by well separated peaks between ammonia and arginine. Arginine gives a low broad peak which is sometimes difficult to plot especially in the analysis of the mother samples, and this restricts the number of readings of this amino acid presented below.

The precision of the analyzer used was constant at 100 ±3% according to repeated check-ups with standard solutions. In all 39 runs an internal standard for each column (AGB and n-leucine) was added to the deproteinized sample prior to analysis on the columns.

In a series of 7 samples where AGP had been added to the plasma as an internal standard prior to deproteinization, the

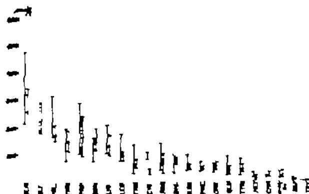


Fig. 3. Mean and range for the individual amino acids of the non-pregnant women (dotted lines) as compared to the range of the adult levels in the literature (solid lines).

TABLE 1 *Error for the whole procedure of deproteinization deproteinization storage and chromatography expressed in per cent of expected adsorption units in 7 samples where AGP was added to the undeproteinized plasma*

| Sample and sample no. | Adsorption units | | Error |
|---|---|---|---|
| | Expected | Achieved | % |
| Mother 420 | 8.93 | 8.75 | −2 |
| Mother 421 | 8.93 | 9.37 | +5 |
| Cord 422 | 8.93 | 9 06 | +1 |
| Non-pregn. 423 | 13 43 | 13.80 | +3 |
| Non-pregn. 424 | 13.42 | 13.71 | +2 |
| Non-pregn. 428 | 8.93 | 9.21 | +3 |
| Non-pregn. 429 | 8 93 | 9 41 | +5 |

samples were analyzed shortly after a fresh standard containing AGP had been run through the analyzer The results are shown in Table 1 giving an error for the whole procedure of ±5%. This is in accordance with Butterfield & O'Brien [7], ±6%, and Dickinson et al. [19], ±5%, who used similar methods.

## Material

The sex-difference in the plasma amino acid levels is known [30, 41], women having significantly lower level than men. Infor mation in the literature bout the plasma amino acid levels in adult women is limited and in order to be ble t characterize the free amino acid levels of the mothers in our material, plasma from 8 fasting non preg nant women f fertile age was analyzed In each case the woman general health was known to the authors. Their weight and height were within standard deviation for age according to Boe et al [5] except for one woman who was 7 cm above +1 S.D., but showed normal weight for her height, and for one woman whose weight was 3 kg below −1 S.D

Plasma from the mother and the cord was collected at 10 deliveries. The mother number of pregnancies and the age-length weight distribution are shown in T ble 2. The mother weight and height before pregnancy were within standard deviation for age [5], except for 2 mothers whose weights were 3 and 5 kg respectively below −1 S.D They were all subjectively healthy and had no history of previous disease All had a normal crown vaginal delivery after a normal uneventful gest tional period. The mother blood pressure during pregnancy and labour was less than 140/90 and they showed no protein or glucose in their urine All the mothers were Rh-positive and no child showed hyperbilirubinemia. The maxi

TABLE 2 *Age weight before pregnancy height and number of pregnancy of the mothers Length of gestational period and weight crown-heel length and sex of the child at birth*

| Mother | | | | Child | | | |
|---|---|---|---|---|---|---|---|
| Age in yrs | Weight in kg | Height in cm | No. of pregn. | Gestat weeks | Weight in g | Length in cm | Sex |
| 18 | 48 | 164 | II | 43 | 3070 | 47 | boy |
| 18 | 54 | 161 | I | 40 | 2730 | 47 | girl |
| 19 | 58 | 165 | I | 37 | 3030 | 49 | boy |
| 26 | 47 | 158 | II | 39 | 3220 | 49 | girl |
| 28 | 60 | 166 | II | 41 | 3890 | 51 | boy |
| 32 | 54 | 160 | I | 39 | 3000 | 48 | girl |
| 33 | 63 | 163 | IV | 41 | 4600 | 52 | girl |
| 34 | 65 | 171 | V | 41 | 3770 | 51 | girl |
| 38 | 62 | 169 | II | 40 | 3460 | 49 | girl |
| 40 | 62 | 167 | V | 40 | 4010 | 52 | boy |

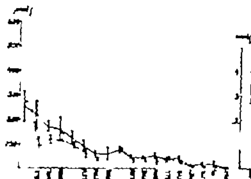


Fig 4 Mean and ±1 S.D for the individual amino acids plus urea in the mothers (dotted lines) as compared t the mean and ±1 S.D for the same compounds in the non-pregnant women (solid lines)

inal total length of deli very was 24 hours in the primiparae and 9 hours in the multiparae The anaesthesia used was nitrous oxide for 1 2 hours before delivery in 5 cases, and 5–10 g of chloroform during deli very in 6 cases There was no sign of intra or extra-uterine asphyxia, all the children showed an Apgar score of 10 point at one and at four minutes after delivery In no case was the cord around the neck of the child or otherwise obstructed All the children had a weight and a crown heel length within ± S.D for gestational age and sex [21]. At examinations by one of us during the neonatal period they showed no signs f immaturity or postmaturity and a normal weight increase after less than 10% initial weight loss.

The placentas were all normal upon examination by one of us after deli very Their weight was within the standard deviation for gestational period according to Hendricks [48]

## Results

The range mean and standard deviation for the individual amino acid levels are expressed in μmol/l plasma in Table 3 The number of successful estimations of each amino acid in the different groups is indicated (n) The ratio between the cord level and that of the mother s cubital vein was estimated in each pair and the values for range mean and standard deviation for these ratios are given in Table 3

The mean and standard deviation for the individual amino acids in the mothers are compared to those of the non pregnant women in Fig 4 The order from left to right of the amino acids in these "aminograms is according to decreasing levels in the normal adult s venous plasma as proposed by Soupart [51] If the mean values for all amino acids studied are added the total concentration amounts to 22.0 mg/100 ml in the non pregnant women and 1 0 mg/100 ml in the mothers The more than 3-fold increase of the *alanine/glycine-quotient* in the pregnant women s venous plasma at delivery is apparent in Fig 4 Besides this the venous plasma of the mothers seems to be characterized by a comparatively low *glycin* and *arginine* level.

The aminogram of the cord plasma is compared to that of the mother s plasma in Fig 5 A general increase of all the amino acids studied is seen in the cord plasma giving a total concentration of 32.8 mg/100 ml and an over-all ratio of 1 9/1 The cord venous plasma at delivery seems to be characterized by a comparatively high level of *lysine* and *taurine*.

The ratios of the cord vein levels to the mother s venous levels show a great variation for the different amino acids (see Fig 6 ) All show a higher ratio than 1 0 The *arginine* level of the mothers is universally very low giving a lim ted number of possible estimations and the ratios show a very great standard deviation

TABLE 3. *Free amino acid levels in venous plasma from normal non-pregnant women, the mother during delivery and the cord during delivery with ratios between the cord vein level and that of the mother's cubital vein*

Range, mean and standard deviation are expressed in µmol/l plasma. Mean is also given in mg/100 ml plasma (in brackets). n = number of cases where the amino acid was successfully determined. Compounds below dotted line are those which are better determined with other methods.

| Amino acid | Non-pregnant women | | | | Mothers | | | | Cord blood | | | | Ratio cord/maternal plasma | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Range | n | Mean | s.d. | Range | n | Mean | s.d. | Range | n | Mean | s.d. | Range | n | Mean | s.d. |
| α-Alanine | 168–341 | 8 | 255 (2.3) | 61 | 280–490 | 10 | 339 (3.0) | 67 | 324–601 | 10 | 441 (3.9) | 75 | 1.0–1.8 | 10 | 1.3 | 0.2 |
| Glycine | 161–323 | 8 | 223 (1.7) | 55 | 64–160 | 10 | 94 (0.7) | 25 | 206–298 | 10 | 239 (1.8) | 32 | 1.8–3.5 | 10 | 2.5 | 0.6 |
| Valine | 153–211 | 7 | 174 (2.0) | 21 | 81–140 | 10 | 121 (1.4) | 18 | 169–272 | 10 | 224 (2.6) | 28 | 1.5–2.4 | 10 | 1.9 | 0.3 |
| Proline | 94–240 | 8 | 162 (1.9) | 48 | 88–168 | 10 | 115 (1.3) | 22 | 119–208 | 10 | 160 (1.8) | 28 | 1.2–2.5 | 10 | 1.5 | 0.4 |
| Lysine | 97–171 | 9 | 130 (1.9) | 27 | 84–114 | 10 | 99 (1.5) | 12 | 277–388 | 10 | 318 (4.6) | 33 | 2.5–4.1 | 10 | 3.3 | 0.7 |
| Leucine | 77–111 | 8 | 96 (1.3) | 22 | 40–83 | 10 | 64 (0.8) | 17 | 82–198 | 10 | 118 (1.6) | 33 | 1.3–2.9 | 10 | 1.9 | 0.5 |
| Taurine | 51–94 | 9 | 64 (0.8) | 11 | 29–60 | 10 | 42 (0.5) | 11 | 97–272 | 10 | 161 (2.3) | 58 | 2.0–5.7 | 10 | 4.2 | 1.0 |
| Arginine | 20–101 | 8 | 62 (1.1) | 26 | 15–33 | 4 | 22 (0.4) | 8 | 37–135 | 9 | 65 (1.1) | 30 | 2.2–5.5 | 3 | 3.4 | 1.9 |
| Histidine | 64–107 | 9 | 79 (1.2) | 13 | 63–93 | 4 | 79 (1.2) | 8 | 93–135 | 4 | 112 (1.7) | 17 | 1.2–1.8 | 4 | 1.6 | 0.3 |
| Isoleucine | 41–63 | 8 | 49 (0.6) | 8 | 23–46 | 10 | 34 (0.5) | 8 | 42–90 | 10 | 62 (0.8) | 12 | 1.3–2.6 | 10 | 1.9 | 0.4 |
| Phenylalanine | 41–54 | 8 | 46 (0.8) | 5 | 23–44 | 10 | 28 (0.6) | 7 | 55–90 | 10 | 67 (1.1) | 10 | 1.3–2.6 | 10 | 1.9 | 0.4 |
| Ornithine | 41–70 | 9 | 58 (0.7) | 11 | 19–47 | 6 | 29 (0.4) | 11 | 60–121 | 8 | 89 (1.2) | 20 | 2.0–4.6 | 6 | 3.3 | 1.0 |
| Glutamic acid | 21–52 | 8 | 38 (0.6) | 11 | 34–54 | 10 | 44 (0.7) | 7 | 46–128 | 10 | 68 (1.0) | 24 | 0.9–3.4 | 10 | 1.6 | 0.7 |
| Tyrosine | 34–63 | 8 | 45 (0.8) | 10 | 16–32 | 10 | 26 (0.5) | 5 | 42–83 | 10 | 61 (1.1) | 14 | 1.7–3.4 | 10 | 2.4 | 0.6 |
| Methionine | 6–19 | 8 | 13 (0.2) | 5 | 4–12 | 9 | 8 (0.1) | 3 | 10–22 | 10 | 18 (0.3) | 6 | 1.5–3.8 | 9 | 2.4 | 0.7 |
| Citrulline | 12–31 | 8 | 20 (0.4) | 7 | 5–16 | 9 | 8 (0.1) | 4 | 5–15 | 9 | 9 (0.2) | 3 | 0.8–1.6 | 8 | 1.1 | 0.4 |
| α-$NH_2$-BU | 11–30 | 4 | 21 (0.2) | 12 | 6–11 | 3 | 9 (0.1) | 3 | 15–31 | 5 | 24 (0.2) | 7 | 1.8–3.6 | 3 | 2.6 | 1.1 |
| Aspartic acid | 1–7 | 9 | 4 (0.1) | 3 | 2–7 | 10 | 5 (0.1) | 3 | 4–20 | 9 | 10 (0.1) | 5 | 1.3–3.7 | 9 | 2.0 | 0.8 |
| Urea | 2670–4610 | 9 | 3370 (20.4) | 646 | 1290–3210 | 10 | 2220 (13.0) | 670 | 1530–3440 | 10 | 2590 (15.5) | 610 | 0.9–1.4 | 10 | 1.1 | 0.1 |
| Threonine | 112–206 | 4 | 145 (1.7) | 42 | 92–144 | 3 | 124 (1.5) | 20 | 209–262 | 3 | 234 (2.8) | 26 | 1.6–2.3 | 3 | 1.9 | 0.4 |
| Serine | 117–197 | 4 | 147 (1.5) | 37 | 58–101 | 3 | 72 (0.8) | 24 | 127–133 | 3 | 130 (1.4) | 3 | 1.3–2.2 | 3 | 1.9 | 0.5 |
| Cystine | 27–46 | 6 | 37 (0.9) | 7 | 29–40 | 4 | 36 (0.9) | 10 | 33–43 | 5 | 38 (0.9) | 5 | 1.0–1.2 | 3 | 1.1 | 0.1 |
| Glutamine | 555–792 | 4 | 716 (10.5) | 115 | 264–376 | 3 | 331 (4.8) | 47 | 492–590 | 3 | 550 (8.0) | 58 | 1.5–2.0 | 3 | 1.7 | 0.3 |
| Asparagine | 53–94 | 4 | 74 (1.0) | 17 | 38–68 | 3 | 51 (0.7) | 16 | 53–66 | 3 | 59 (0.8) | 4 | 1.0–1.5 | 3 | 1.3 | 0.3 |
| Total amino acids | | | (22.6) | | | | (17.0) | | | | (32.5) | | | | 1.9 | |

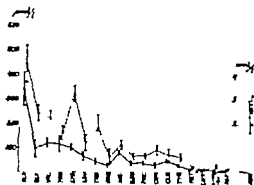


Fig. 5. Mean and ±1 s.d. for the individual amino acid plus urea in the cord plasma (dotted lines) as compared to the mean and ±1 s.d. for the same compounds in the mothers (solid lines).

from the high mean value. Especially high ratios were estimated for *lysine, taurine* and *ornithine*.

## Discussion

The vast literature on the subject of placental amino acid transfer has been reviewed by Needham [38], Schreier & Plückthun [46, 48, 49], Dancis [15, 16] and Waisman & Kerr [54]. In view of all the known influences on the plasma amino acid pool thorough clinical correlation seems most important in investigations concerning the placental transfer of amino acids. Such known influences are liver and kidney disease, stress, and hormonal disturbances. The alpha-amino-nitrogen level of cord blood depends on the length of the gestational period [31]. Toxicosis of pregnancy [30, 13, 10, 12, ] and umbilical blood flow interruption [12] seem to influence the foetal/maternal amino acid ratio. The nutritional status and age of the mother, the number of previous pregnancies, disease or anomaly of the placenta and asphyxia are all factors which may well have an influence on the amino acid levels of mother and child. The normal individual variation, different for the individual amino acids studied, is great according to Iob *et al.* [29]. An inter individual variation of 30% and an intra individual variation of 10% was demonstrated.

No attempt to explain the normal amino acid levels will be made, but some facts that might be of physiological importance for the characteristic pattern will be mentioned. The difference found between the pregnant women at term and the non-pregnant women are in accordance with earlier findings [11, 22]. The lower level of most amino acids can be at least partly explained by the increase in blood volume towards term. Both Christensen [11] and Furuya & Machara [22] could show a higher level of plasma amino acids in the mother during labour than during pregnancy. The time for equilibrium over the placental membranes is known to be approximately 30 minutes in the rabbit [6]. Thus our results from the mother's plasma and the cord plasma during deli-



Fig. 6. Mean and ±1 s.d. for the ratios of the cord vein amino acid level to that of the mother's cubital vein. The mean values are all above the solid line at 1.0. The over-all ratio of 1.9 is indicated with the dotted line.

very cannot be taken as representative of the conditions in late pregnancy

The comparatively high cord plasma levels of lysine and taurine are in accordance with earlier reports (microbiological method, [4 22] paper chromatography [58, 30] and ion exchange chromatography [20 25 4]) The pattern of the cord plasma aminogram agrees with that of the 8 cases of Ghadimi & Pecora [24] except for alanine which shows a higher level both in the mother and the cord blood, and glutamic acid, which shows a lower level

It is known that during the first 5-6 days of life the normal newborn excretes increased amounts of amino acids predominantly taurine in its urine (reviewed by Dustin [20]) This might be a result of an overflow reaction in the partly immature kidney Taurine is used in the formation of biliary acids and the high plasma level of taurine could be a sign of decreased uptake or metabolism of this amino acid by the foetal liver Lysine is a unique amino acid since it takes no part in transamination processes [55] Many enzymes associated with degradation of amino acids have a low activity in the foetal liver at term [17] Normally lysine is rapidly incorporated into the liver [34] and the increased plasma level t birth could be a result of the low blood flow in the foetal liver [48] The potassium level of cord plasma is increased [56]. Intracellular lack of potassium gives an increase of basic amino acids in plasma [10]. Nicolaidau *et al* [30] has proposed that cord cells have an intrinsic property that gives a high concentration of basic amino cids in cord plasma. In our investigation the increase of the other basic amino acids is not comparable to that of lysine

Cord blood shows hyperaminoacidemia even when compared to the normal adult levels, but this is not uniform. Proline serine and citrulline show like glutamine asparagine and urea lower levels than in adult plasma. The fact that the artificial increase of one amino acid in plasma can give a general hyperaminoacidemia could be of importance in the normal cord plasma pattern. The specific optimal requirements of each amino acid might vary during different phases of growth as shown in the mosquito (Culex Pipiens L.) by Chen & Briegel [8].

By using the manometric method Christensen & Streicher [9] and Clemetson & Churchman [1 ] estimated the foetal/maternal amino acid ratio to be 1.7/1 in favour of the foetus The general increase of all amino acids in the cord plasma compared to the mother s plasma with the especially high ratios for lysine and taurine are in accordance with earlier findings (paper chromatography [13] and ion exchange chromatography [25 4]) The only comparable figures in the literature concerning our total medium concentration of 17 0 mg/100 ml in the mothers and 3..8 mg/100 ml in the cord plasma and a total ratio of 1.9/1 are those of Ghadimi & Pecora (8 cases [ 4]) with 19 6 and 33.8 mg/100 ml respectively giving an over-all ratio of 1 7/1. The especially high ratios for ornithine and arginine, in addition to those for lysine and taurine were also described by Butterfield & O'Brien [7] and Ghadimi & Pecora [ 4] These might be explained by the low need for urea-formation during the positive nitrogen balance of intensive intrauterine growth [32] There is a possible competition between arginine and lysine [3].

## Summary

The characteristic plasma amino acid pattern of pregnant women during delivery is compared with that of non pregnant women of fertile age. The range mean and standard deviation for 18 free amino acid are given. The pregnant woman at delivery seems to have an increased plasma alanine/glycine quotient and a comparatively low plasma glycine and arginine level. The amino acid pattern of cord plasma is compared to that of the mother s plasma at delivery and range mean and standard deviation for the 18 free amino acids studied are given. Cord plasma is characterized by hyperaminoacidemia with a marked increase of the lysine and taurine levels. The ratios between the cord plasma level and those of the mother s cubital vein are given for the individual amino acids with range mean and standard deviation. Lysine taurine ornithine and arginine show especially high ratios (more than 3/1) while the over-all ratio was estimated to be 1.0/1 in favour of the cord plasma

The importance of thorough clinical correlation is stressed in view of all the normal and abnormal factors influencing the plasma amino acid pool. The characteristic normal patterns are discussed.

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# Intolerance to Cow's Milk and Wheat Gluten in the Primary Malabsorption Syndrome in Infancy

by J. K. VISAKORPI and PIRKKO IMMONEN

Since 1950, when Dicke *et al.* [5] reported their observation on the harmful effect of gluten on patients with coeliac disease, the main interest in the study of this disease has been focused on the pathogenetic role of gluten. Persistent sensitivity to gluten has even been taken as a diagnostic criterion in coeliac disease. However, absorption defects and other coeliac symptoms have been described in some patients in whom the disease was undeniably related to cow's milk [4, 12]. Especially the report of Fällström *et al.* [6] gives interesting new information, suggesting that cow's milk intolerance may pave the way for a later developing sensitivity to gluten, as proposed earlier by Heiner *et al.* [8].

The malabsorption syndrome in childhood has been under investigation in our hospital during the past few years. The occurrence of the various types of malabsorption syndrome and significance of absorption tests in the diagnosis of these diseases have been presented in another paper [15]. Infants with intolerance to cow's milk and/or gluten constituted the largest and therapeutically most important group in this series. In the present paper we will describe clinical observations on food intolerances in these patients.

## Material and Methods

During the three year period 1962–1965 every patient admitted to the Children's Hospital, University of Helsinki, because of symptoms suggestive of malabsorption (abnormal stools and/or failure to thrive) was subjected to a similar series of absorption tests. These included faecal fat determination, D-xylose excretion test, FIGLU test, barium meal, sweat test, disaccharide loading test, measurement of pancreatic enzymes and immunodiffusion test for antibodies against cow's milk and gluten. These tests were performed as described elsewhere [15].

The patients in whom absorption defects were verified, but special diseases which may cause malabsorption excluded, were submitted to a therapeutic trial with elimination diets. The patients were at first observed on their regular diets for at least two weeks, after which, if no improvement took place, a gluten free diet was instituted. If there was no improvement on this regimen, breast milk was started. Patients who had never received wheat-containing food were treated with breast milk after the observation period. In some cases, elimination diet was instituted immediately because of the critical state of the patient. When the patient was showing clinical improvement on the elimination diet, provocation tests were

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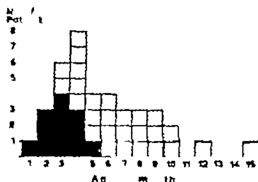


Fig. 1. Ages of the patients at onset of symptoms. Black columns = patients with cow's milk intolerance. White columns = patients with gluten intolerance.

performed. The time-table of the provocation tests was variable. Diagnosis of intolerance was based on the favourable effect of elimination diet alone.

The provocation test with cow's milk consisted of giving 100–200 g of two-third strength cow's milk once. If no reaction was observed, the patient was gradually transferred to regular cow's milk feeds. Some of the patients who reacted to cow's milk were also tested with purified commercial bovine milk proteins. A single meal of 3 % wheat water mixture was used to test wheat sensitivity in patients intolerant to cow's milk. In other patients this provocation was done with 100–200 g of wheat milk mixture. If no reaction ensued, a wheat-containing diet regular for the age was instituted. Sensitivity to maize was also tested in many of the patients. The test meal was prepared similarly to that for wheat.

## Description of the Patients

Of the 40 infants with the primary malabsorption syndrome, 12 exhibited intolerance to cow's milk, and 28 patients were intolerant to gluten. Fig. 1 demonstrates the ages of patients when symptoms appeared and Table 1 shows the most important clinical features of these patients. Admission to hospital occurred on the average 1–months after the onset of symptoms. The symptomatology of these patients was relatively uniform consisting mainly of abnormal stools, vomiting and failure to gain weight. Other symptoms were rare in these patients. Symptoms typical of coeliac disease such as "coeliac stools" and enlarged abdomen, were more common in the gluten intolerant group. The high frequency of "regular" watery diarrhoea in this series should be noted.

Absorption defect was a constant finding, but routine laboratory tests usually gave normal results. Megaloblastic anaemia was not found and hypochromic anaemia occurred in a few cases. Serum iron, however, was low in many cases especially in older infants. Serum calcium was low in two cases but rickets was not observed. Marked hypoproteinaemia was also a rare finding. Duodenojejunal biopsy almost without exception showed more or less severe villous atrophy. The patients with gluten intolerance usually had severer changes. The results of absorption tests [18], immunological tests [9] and duodenojejunal histology [10] are discussed in greater detail elsewhere.

## Intolerance to Cow's Milk and Gluten

### *Effect of Elimination diets*

Patients who recovered when cow's milk was eliminated from the diet usually exhibited a uniform response to the elimination. Vomiting was the first symptom to disappear, ceasing in a few days; watery diarrhoea stopped on the average in a week, and the patient started gaining weight in 4–4 weeks. There was only one exception to this pattern. In this case (P. H.) the diarrhoea started very early at the age of weeks, and the patient only recovered after 3 months on breast milk.

In patients who responded to gluten-free diet, recovery was usually slower. Vomiting ceased in 1–2 weeks, watery

TABLE 1 *Essential symptoms signs and findings in infants with the primary mal absorption syndrome*

| | Patients with | |
|---|---|---|
| | cow milk intolerance | gluten intolerance |
| Numbers of patients | 12 | 28 |
| ***Symptoms*** | | |
| Watery diarrhoea | 9 | 17 |
| Otherwise abnormal stools (pale greasy offensive) | | 9 |
| Vomiting | 10 | 22 |
| Failure to gain weight | 12 | 28 |
| Failure to grow | 3 | 10 |
| ***Signs*** | | |
| Enlarged bdomen | 6 | 21 |
| Bleeding | 0 | 1 |
| Oedema | 0 | 1 |
| ***Findings*** | | |
| F t excretion, more than 4 g/day | 11 | 25 |
| D xylose excretion, less than 15 | 7/11 | 23 |
| Positive urinary INDICAN | 8/10 | 24/27 |
| Haemoglobin less than 10.0 g/100 ml | 2 | 6 |
| Serum iron, less than 40 μg/100 ml | 3 | 18 |
| Serum protein, less than 5 g/100 ml | 1 | 3 |
| Serum calcium, less than 4 mEq/l | 1 | 1 |
| Lactosuria, more than 100 mg/100 ml | 4 | 7/23 |
| Saccharosuria, more than 100 mg/100 ml | 2 | 5/23 |
| Faecal culture positive for Staph. aureus | 5 | 8 |
| Faecal culture positive for enteropathogenic E. coli | 0 | 2 |
| Precipitins to cow milk | 8/10 | 22/23 |
| Precipitins to gluten | 8/10 | 16/23 |
| Barium meal, "malabsorption pattern | 7/10 | 21/23 |
| Normal duodenojejunal mucosa | 0/10 | 1/15 |
| Slight changes or partial villous atrophy | 5/10 | 3/15 |
| Subtotal villous atrophy | 5/10 | 11/15 |

The number of patients studied is indicated separately in the table when not all have been investigated.

diarrhoea when present, disappeared in 1–3 weeks, and it took up to 3–4 weeks before the patient began to gain weight.

*Cow s milk intolerance*

T ble 2 shows the results of provoca tion tests in patients intolerant to cow milk. It can be seen that clinical tolerance to cow milk appeared in most cases at the same age (7–10 months) regardless of the duration of the elimination diet The same patient (P H ) as mentioned before showed unusually prolonged intolerance.

Typically a positive provocation with cow milk rapidly (in *–4 hours) resulted in profuse vomiting with or without diarr hoea (8 cases) Two patients reacted with diarrhoea only One patient, in addition exhibited fever reaction. Provocations with pure milk protein fractions were per

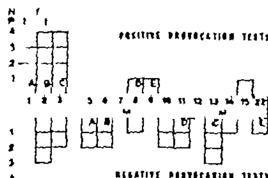


Fig. — Result of provocation tests in patients with gluten intolerance. Block letters indicate five patients in whom both positive and negative tests were obtained.

formed in 4 cases (Table 3). The patients reacted to several of the proteins tried. Patient P H manifested an unusual sensitivity to beta lactoglobulin, reacting with vomiting to 10 mg of this protein in breast milk.

*Gluten sensitivity in patients intolerant to cow's milk*

In Table 2 these patients are divided into three groups according to whether they had received gluten-containing food before the onset of symptoms, during the disease or not at all. Altogether 8 of the 12 patients proved to be sensitive to gluten as well. Three of these had never received gluten before. Gluten intolerance was demonstrable at the same period as cow's milk intolerance and continued in 4 cases for even longer than that. Positive reactions to maize were not obtained.

The reaction to gluten was rapid. In 4 cases there was vomiting and diarrhoea, in 2 cases only vomiting and in 2 cases only diarrhoea. In addition, one patient had fever and two patients exhibited a severe anaphylactic reaction with collapse. These severe reactions occurred in patients who had been fed with gluten before the onset of symptoms.

*Gluten sensitivity in patients intolerant to gluten only*

Of the 23 patients recovering on a gluten free diet, 14 reacted to provocation

TABLE 2. *Results of provocation tests in patients with cow's milk intolerance.*

| Cases | | Age at onset of elimination diet (months) | Provocations with Cow's milk: Age at last positive provocation (months) | Cow's milk: Age at first negative provocation (months) | Gluten: Age at positive provocations (months) | Gluten: Age at negative provocations (months) |
|---|---|---|---|---|---|---|
| Patients who had never received wheat | MH | 3 | 8 | 12 | | 4, 5, 6½, 8, 17 |
| | PK | 3½ | 8 | 7 | 5, 8½ | 8, 13, 17 |
| | VI | 6½ | 6 | 8 | 6, 9½ | 18, 19 |
| | RR | 2½ | 7 | 11 | | 4, 5, 9, 11, 13 |
| | PH | 2 | 15 | | 10 | |
| Patients who had received wheat after onset of symptoms | TH | 4 | 8 | 10 | 14 | 17 |
| | PH | 3½ | 5 | 7 | 5 | 15 |
| | JM | 3 | 3½ | 6½ | | 6, 9 |
| Patients who had received wheat before onset of symptoms | KP | 6½ | | 8½ | | 13, 41 |
| | SN | 5½ | 6½ | 7½ | 5, 9 | |
| | EL | 4 | 7 | 10 | 5, 7, 10 | |
| | ML | 5 | 6½ | | 7 | |

TABLE 3. *Reactions to cow's milk and some bovine milk protein fractions in 4 patients with cow's milk intolerance.*

| Patient | Whole milk 100 g | Casein 2800 mg | Lactalbumin 100 mg | Beta-lacto-globulin 320 mg | Bovine serum albumin 40 mg | Bovine gamma-globulin 60 mg |
|---|---|---|---|---|---|---|
| | Proteins used in provocations, amounts in mg* | | | | | |
| RR | + | + | − | + | − | − |
| EL | + | + | − | − | − | − − |
| MH | + | | | + | − | |
| PH | + | + | | + | + | − |

Casein was obtained from Hoffman La Roche lactalbumin and bet lactoglobulin from Nutritional Biochemicals Co., bovine albumin powder and bovine gammaglobulin from the Armour Pharmaceutical Company Ltd. The doses correspond roughly t the content of protein in 100 g whole milk.

tests and 13 did not react when tested after 2 to 13 months on elimination. One patient is still on an elimination diet without provocation. The duration of the clinical intolerance to gluten was variable, and our observations do not give exact information on this point. As repeated provocations may be dangerous to the patient they were not performed systema tically In only 5 patients were both positive and negative provocation tests obtained. These patients are indicated by capitals in Fig .. This figure demonstrates that many patients still showed gluten sensitivity after 1–3 months elimination diet, but in several other patients sensiti vity had disappeared in a few months. In most cases tolerance to gluten developed during the first year after institution of elimination diet. One patient was still intolerant after 15 months. Another pa tient seemed to tolerate gluten after 22 on elimination diet but then .apsed three months after resumption of a normal diet. The patients have been .owed up for 6 months to 4 years and no other relapses have been observed. The duration of intolerance is apparently unrelated to the age of the patient and also to the duration of symptoms before treatment in this series.

In provocation tests with gluten, only 6 out of 14 patients reacted rapidly to a single gluten-containing meal with vomit ing and diarrhoea (4 patients) or with vomiting only (* patients) In one of these anaphylactic collapse occurred. Eight patients reacted only when a gluten-con taining diet was continued. Intolerance was manifested in these cases on the average in two weeks (range 1 to 3 weeks) with reappearance of all the symptoms of the disease diarrhoea vomiting and failure to gain weight.

*Relationship between intolerance and absorption function*

Provocation tests were performed when the patient was clinically clearly improv ing. Even patients in very good clinical condition showed strong intolerance Every patient had abnormal absorption

tests when the elimination diet was started, and these usually normalized simultaneously with the disappearance of the intolerance. Of the patients whose absorption function was followed up by repeated testing, 8 had normal absorption test results at the time of the first negative provocation. In two of these absorption function was already normal before tolerance had developed. Three patients still showed signs of absorption defects when the provocation test was negative.

## Conclusions and Discussion

Forty infants with the primary malabsorption syndrome were diagnosed during a three-year period at our hospital. In about one-third of them intolerance to cow's milk was observed, in most cases associated with intolerance to gluten, the remaining patients being intolerant to gluten only.

The symptomatology of these patients contained many typical coeliac symptoms but some differences were also seen. Watery diarrhoea was more common than typical coeliac steatorrhea, especially in patients with cow's milk intolerance. Vomiting was a rather common symptom but enlarged abdomen was not so prominent as in the older coeliacs. The young age of the patients and short duration of the disease may explain these differences. Absorption defects and small intestinal villous atrophy of variable degree were constant findings. There was no clear difference in symptoms, signs or results of laboratory investigations between patients intolerant to cow's milk and gluten. This fact points to a close relationship between these two groups of patients, and we feel that it is not correct to separate these two forms of the primary malabsorption syndrome under such different titles as milk allergy and coeliac disease.

Among the 1? infants with cow's milk intolerance 8 patients had not received gluten before the onset of the disease. Thus any aetiological role of gluten is excluded in these cases. This fact demonstrates that coeliac symptoms and villous atrophy of the intestinal mucosa are not always associated with gluten induced disease, as has already been pointed by Kuitunen *et al.* [11].

Intolerance to cow's milk seems to be bound to a restricted age period. Tolerance usually developed between 7–10 months, as also in the patients described by Fällström *et al.* [6]. Those patients with cow's milk intolerance who had received gluten in their diet demonstrated clear intolerance to gluten also. This still continued in many cases after the patient had become tolerant to cow's milk. This fact is further evidence corroborating the suggestion made by Heiner *et al.* [8] and subsequently supported by Fällström *et al.* [6] that cow's milk intolerance may set the stage for gluten intolerance. An unexpected phenomenon was that three patients who had never taken wheat or other cereals reacted clearly, although not strongly, to wheat when tested for the first time. This may be interpreted as a direct toxic effect of gluten on the damaged intestinal mucosa.

Disaccharide malabsorption is a well documented cause of cow's milk intolerance and has been established to be a factor contributing to the diarrhoea in the primary malabsorption syndrome [1]. Burke *et al.* [.] describe a series of patients very similar to ours, in whom disaccharide malabsorption was the cause of cow's

milk intolerance. In our patients this is apparently not the case. Our patients recovered well with breast milk, which contains about the same amount of disaccharide as the cow's milk formula they did not tolerate. In addition, our patients reacted strongly in provocation tests to rather small doses of cow's milk or purified proteins of cow's milk in breast milk. In some cases, secondary disaccharide malabsorption may play a minor role.

The duration of gluten intolerance in coeliac disease has been the subject of many discussions. Especially in older children, long-continued intolerance has been described as typical and even diagnostic. On the other hand, some authors [3, 13] have included in their series of coeliac cases patients whose intolerance was of short duration. Grüttner wants to separate these cases from true coeliac disease calling them abortive forms [7]. Our study clearly shows the great variations in the duration of intolerance. In most cases this was surprisingly short. The intolerance period was estimated on a clinical basis only, however, and in some cases in which intestinal mucosal histology was followed [10] it was seen that mucosal changes persisted after clinical tolerance had already developed. In only [illegible] cases was intolerance of more than one year's duration verified, and in 4 other patients it was possible. No correlation was seen between the age of onset or length of disease before treatment and the duration of intolerance. Sheldon & Simpkiss [14] have drawn the same conclusion from their series.

Our results demonstrate that infants with the primary malabsorption syndrome often exhibit intolerance to food proteins (especially to cow's milk and gluten) and respond well to elimination diets. The intolerance is apparently of shorter duration than that described in older children and adults with coeliac disease. The explanation of this difference may be that the aetiology of the disease is heterogeneous. In patients with persistent intolerance the disease may be due to a congenital defect, whereas in patients with intolerance of short duration the harmful effect of gluten might be secondary and only manifests when the intestinal mucosal barrier is primarily damaged by some other factor such as infection or allergic reaction.

## Summary

Intolerance to cow's milk and gluten was studied in 40 infants suffering from the primary malabsorption syndrome. The clinical picture of the patients was relatively uniform, consisting of diarrhoea, vomiting, failure to gain weight, absorption defects and villous atrophy of the intestinal mucosa.

Cow's milk intolerance was verified in 12 patients, 8 of whom were also intolerant to gluten. In 8 cases the first symptoms had appeared before wheat was incorporated into the diet. Tolerance to cow's milk usually developed between the ages of to 10 months. The associated intolerance to gluten disappeared during the same period in 4 cases, and persisted a few months longer than cow's milk intolerance in 4 patients.

In patients intolerant to gluten only (28 cases) the length of the intolerance period was very variable. In many cases the intolerance disappeared in a few months, and only a few patients showed prolonged intolerance.

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# Adenovirus Type 5 Infections

## *An Outbreak of Febrile Pharyngitis in a Home for Infants*

*by* SIGVARD WOLONTIS GÖSTA TUNEVALL and GÖRAN STERNER

It is well known that adenovirus types 1 and 5 are common among infants and small children [1 2, 4, 5 9 11–1 22]. However the association of these types of adenovirus with illness has been debated. Sutton [21] and Gardner [7] for instance could not find any correlation between the isolation of adenovirus type 5 and acute respiratory illness in nursery children. Bell *et al.* [*] in a three-year investigation of nursery children found that, among 16 different types of adenovirus isolated, only types 1 3 and 5 were definitely correlated with febrile illness. One of our previous studies [16] in a childrens hospital over a one year period showed that in children aged 6 months to 3 years the excretion of adenovirus types 1 2 and 5 was significantly correlated to the presence of acut respiratory illness on admission.

This report will deal with an outbreak of febrile pharyngitis in a home for infants May 1965, during which we were able to demonstrate the presence of denovirus type 5 infection. Using virological and bacteriological methods including bacterial cultures, attempts at virus isolation and serological tests against a number of antigens (viruses Mycoplasma pneumoniae and bacteria) it was possible to rule out many of the other infections common in infants.

### Material and Methods

The study was carried out at a home for infants in Stockholm during May and June 1965. During this period 14 infants aged 1–10 months were resident nd there were 12 staff members.

Newborn infants (2–3 weeks old) are admitted to this home from several obstetrical departments in Stockholm. On arrival the newborn is placed in the smallest room No. 2, on the second floor (Fig 1). At the age of 1½–2 months, the infant i moved to room No. 3 also on the second floor and finally transferred to room No 1 on the ground floor

During the outbreak of febrile illness in May 1965 no transfer of infants between the rooms took place. In June only two infant (cases 9 and 13) were moved from room N 1

The head nurse noted temperatures daily plus signs of acut respiratory illness, e.g. coryza and cough and gastrointestinal symptoms, e.g. vomiting and diarrhoea. Clinical examination of the infants was carried out

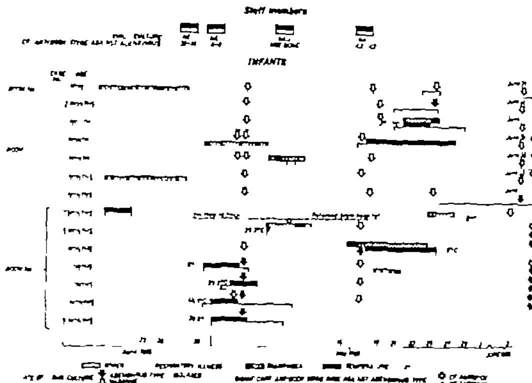



Fig. 1 Acut respiratory illness and it association with adenovirus type 5 infections in home for infants.

by one of us (G S.) once a week and when the infant fell ill.

Fig 1 shows the occurrence of illness among infants and staff members shortly before the observation period and during May 1965 Nine infants and four of the staff fell ill with fever ( 37.4°C) and upper respiratory symptoms. No infant was sent to hospital with this illness. In June none of the infant or the staff was ill with acute respiratory illness. However a few of the infants had a short period of loose stools. One of them was case 8, from which adenovirus type 5 was isolated on Jun 22.

*Virological studies* At the onset of the study nasopharyngeal swabs were taken t the bedside from all infants except two. The swabs were immediately soaked in the fluid of tube cultures of HeLa cells, cells of a continuous line f grivet monkey kidney cells: GMK, AH I (8) and primary cynomolgus monkey kidney cells.

Stool specimens were collected several times from all infants (Table 1) and once from the twelve staff members. For the isolation of virus from stools roller tube cultures of H La and cynomolgus kidney cells were used.

These cell cultures were prepared in oculated and maint ined as previously described [10 24]. The methods for the isola tion and typing of the isolated adenovirus strains are also reported elsewhere [10, 14]. The monkey kidney cell cultures inoculated with material from the nasopharynx of some infants (cases 1 4) were also tested for haemadsorbtion with guinea pig erythrocytes 6 days after inoculation.

Two or three sera were btained from each infant except case 1 The interval between samplings was always at least 14 days (Table 2). Sera were also collected from three out of four staff members, who fell ill.

Sera from the same individual were titra-

Table 1 *F ndings of adenovirus type 5 from nasopharynx (NP) and stools*

ND = not done.

| Case No., age (months), sex | Isolation of adenovirus type 5 | | Case No., age (months), sex | Isolation of adenovirus type 5 | |
|---|---|---|---|---|---|
| No. 1<br>9 m.<br>male | NP<br>Stool | − (May 4)<br>+ (May 4) | No. 9<br>2 m.<br>male | NP<br>Stool | − (May 4)<br>− (May 4)<br>− (May 17)<br>− (June 22) |
| No. 2<br>10 m.<br>male | NP<br>Stool | + (May 4)<br>− (May 3)<br>− (May 17) | No. 10<br>1 m.<br>female | NP<br>Stool | − (May 4)<br>− (May 3)<br>− (May 18)<br>− (June 20) |
| No. 3<br>9 m.<br>female | NP<br>Stool | − (May 4)<br>+ (May 4)<br>− (May 17) | N 11<br>1 m.<br>female | NP<br>Stool | − (May 4)<br>− (May 3)<br>− (May 17)<br>− (June 21) |
| No. 4<br>5 m.<br>male | NP<br>Stool | + (May 4)<br>− (May 4)<br>− (May 17) | No. 12<br>1 m.<br>female | NP<br>Stool | − (May 4)<br>− (May 4)<br>− (May 19)<br>− (June 21) |
| N 5<br>4 m.<br>male | NP<br>Stool | − (May 4)<br>− (May 4)<br>+ (May 17) | No. 13<br>2 m.<br>male | NP<br>Stool | − (May 4)<br>− (May 4)<br>− (May 19)<br>+ (May 25)<br>− (June 21) |
| No. 6<br>5 m.<br>male | NP<br>Stool | ND<br>− (May 9)<br>− (May 17) | No. 14<br>1 m.<br>female | NP<br>Stool | − (May 4)<br>− (May 3)<br>− (May 18)<br>− (May 25)<br>− (June 21) |
| No. 7<br>7 m.<br>male | NP<br>Stool | ND<br>− (June 3) | | | |
| No. 8<br>m.<br>male | NP<br>Stool | − (May 4)<br>− (May 4)<br>− (May 18)<br>− (May 25)<br>+ (June 20) | | | |

ted simultaneously for *complement fixing* (*CF*) *antibodies* against adenovirus. They were also screened at a dilution of 1/8 for CF antibodies against influenza A and B parainfluenza 1 2 and 3, mumps, psittacosis, and respiratory syncytial (RS) viruses. Positive sera were serially titrated from a ½ dilution. In all CF tests the method of Fulton & Dumbell [6] as modified by Svedmyr *et al.* [20] was used. A four fold or greater rise was considered significant

Paired sera were also tested for the occurrence of *neutralizing antibodies* against adenovirus type 5 by the method of Kjellén *et al.* [10].

*Mycoplasma studies* N ttempt was made t isolate Mycoplasma pneumoniae in this study CF tests against Mycoplasma pneumoniae were carried out with a commercial antigen obtained from Robbin Laboratories, Inc., Chapel Hill, N.C., U.S.A.

*Bacteriological studies* Nasopharyngeal and throat swabs were taken from all infants when the study started and also from three of the four sick staff members at the onset of their illness. The swabs were cultured on routine media for bacteria, but not on enriched media. Pneumococci, β-streptococci st phylococci and Haemophilus influenzae were looked for especially

Paired sera were tested for antistreptolysin (AS) antist phylolysin (ASt ) and antipneu

TABLE 2 *Serological response of complement fixing (CF) antibodies and results of neutralization test (NT) against adenovirus type 5*

ND = not done

| Case No., age (months), sex | Adenovirus type 5 isolated | Antibodies against adenovirus type 5 |
|---|---|---|
| No. 1<br>9 m.<br>male | + | CF ND<br>NT ND |
| No. 2<br>10 m.<br>male | + | CF < (May 3)<br>32 (May 18)<br>NT − (May 3)<br>+ (May 18) |
| No. 3<br>9 m.<br>female | + | CF < (May 3)<br>32 (May 18)<br>NT − (May 3)<br>− (May 18) |
| No. 4<br>5 m.<br>male | + | CF <2 (May 3)<br>>64 (May 18)<br>NT − (May 3)<br>+ (May 18) |
| No. 5<br>4 m.<br>male | + | CF < (May 3)<br><4 (June 3)<br>4 (July ")<br>NT − (May 3)<br>− (July ") |
| No. 6<br>6 m.<br>male | − | CF 4 (May 9)<br>8 (June 3)<br>16 (Oct. 11)<br>NT − (May 9)<br>+ (June 3)<br>+ (Oct. 11) |
| No.<br>7 m.<br>male | − | CF 16 (June 3)<br>16 (Aug. 9)<br>NT + + (June 3)<br>+ + (Aug. 9) |
| No. 8<br>2 m.<br>male | + | CF <2 (May 4)<br><2 (June 3)<br><4 (July 2)<br>NT − (May 4)<br>− (July 2) |
| No. 9<br>2 m.<br>male | − | CF <2 (May 18)<br><2 (June 3)<br>4 (July 2)<br>NT − (May 18)<br>− (June 3) |
| No. 10<br>1 m.<br>female | − | CF <2 (May 4)<br><2 (June 3)<br>4 (July 2)<br>NT + (May 4)<br>+ (July 2) |
| No. 11<br>1 m.<br>female | − | CF 2 (May 4)<br><2 (June 3)<br>4 (July 2)<br>NT ± (May 4)<br>− (July ") |
| No. 12<br>1 m.<br>female | − | CF 16 (May 4)<br>8 (June 3)<br>4 (July 7)<br>NT − (June 3)<br>− (July 7) |
| No. 13<br>m.<br>male | + | CF 2 (May 4)<br><2 (June 3)<br><4 (July )<br>NT − (May 4)<br>− (July 2) |
| No. 14<br>1 m.<br>female | − | CF 4 (May 18)<br>2 (June 3)<br>4 (July 2)<br>NT − (May 18)<br>− (July 2) |

molysin (APn) References for these reactions are given elsewhere [19]. A more than two-fold rise in antilysin reactions was considered significant

## Results

*Virological findings* Adenovirus type 5 was the only virus recovered. As shown in Table 1 it was found in 7 out of 14 infants. All virus strains were recovered in HeLa cultures only The first virus strains were obtained on May 4 from four infants, all living in room No 1 Two other infants (case 5 room No. 1 and case 13 room No 3) excreted the same type of virus strains in stools on May 17 and 25

TABLE 3 *The findings in CF tests against a battery of antigens (viruses and mycoplasma) performed on paired sera from 13 infants*

| | *CF antibody titre. Highest value ½ paired sera* | | | | | | |
|---|---|---|---|---|---|---|---|
| Antigen | Titre <8 | 8 | 16 | 32 | >64 | 4 fold rise | 4 fold fall |
| Adenovirus | 7 | 0 | 3 | 2 | 1 | 8 | 1 |
| Influenza A | 13 | | | | | | |
| B | 13 | | | | | | |
| Parainfluenza 1 | L | 1 | | | | | 1[a] |
| 2 | 13 | | | | | | |
| 3 | 11 | 1 | 1[b] | | | | 1 |
| Mumps virus | 12 | 1 | | | | | 1 |
| RS virus | 13 | | | | | | |
| Psittacosis | 10 | 3 | | | | | 3[a] |
| Mycoplasma pneumoniae | 11 | 1 | 1[b] | | | | |

[a] None of these infants was over 2 months. The antibodies were probably of maternal origin.
[b] This infant was 10 months old.

respectively From case 8 (room No 3) virus was not isolated until June 20

Eight out of 13 infants studied showed a four fold rise in CF antibody titre against adenovirus antigen (Table 2) However four of them (cases 5 9 10 and 11) reached a titre of only 4 and did not develop signi ficant titre rise of neutralizing antibodies against adenovirus type 5

Of the six virus excreters studied, four displayed CF titre rises and three had rises in neutralizing antibody as well, one reached a CF titre of only 4 without signi-ficant evidence of neutralizing antibody (Table 2) The remaining two excreters of adenovirus type 5 showed no conversion in either CF or NT On the other hand, no virus was obtained from one patient (case 6 room No. 1) who had clear-cut rises of both types of antibodies

There were no rises in CF antibody titre against other viral antigens used, but seven infants showed a four fold fall of the titre in one or more of the tests. The highest demonstrable titre in these cases was 8. The antibodies found to disappear may have been of maternal origin as they were found only in infants 1–2 months of age (Table 3) Three of these cases had anti-bodies against psittacosis-lymphogranu loma group. Unfortunately it was im possible to study the same antibodies in maternal sera

A CF titre of more than 8 against viral antigens other than adenovirus was only found in a 10-month-old boy (case 2) who had been ill with acute respiratory illness several times before our study started. Besides a rising CF antibody titre against adenovirus he had a CF antibody titre of 16 against parainfluenza type 3 in both sera sampled (Table 3)

No cytophathic agent was obtained from the stools of the twelve staff mem bers and none of the three sick staff mem bers investigated showed any rise of CF antibody titre against adenovirus or against any other viral antigen used. All sera con-tained neutralizing antibodies against ade novirus type 5 but no titre rises occurred.

*Mycoplasma findings* As mentioned above no attempt was made to isolate

TABLE 2 *Serological response of complement fixing (CF) antibodies and results of neutralization test (NT) against adenovirus type 5*

ND = not done.

| Case No., age (months), sex | Adenovirus type 5 isolated | Antibodies against adenovirus type 5 |
|---|---|---|
| No. 1<br>9 m.<br>male | + | CF ND<br>NT ND |
| No. 2<br>10 m.<br>male | + | CF < (May 3)<br>32 (May 16)<br>NT – (May 3)<br>+ (May 16) |
| No. 3<br>9 m.<br>female | + | CF < (May 3)<br>32 (May 16)<br>NT – (May 3)<br>+ (May 16) |
| No. 4<br>5 m.<br>male | + | CF <2 (May 3)<br>>64 (May 16)<br>NT – (May 3)<br>+ (May 16) |
| No. 5<br>4 m.<br>male | + | CF (May 3)<br><4 (June 3)<br>4 (July 2)<br>NT – (May 3)<br>– (July 2) |
| No. 6<br>4 m.<br>male | – | CF 4 (May 9)<br>8 (June 3)<br>16 (Oct. 11)<br>NT – (May 9)<br>+ (June 3)<br>+ (Oct. 11) |
| No. 7<br>7 m.<br>male | – | CF 16 (June 3)<br>16 (Aug. 9)<br>NT + + (June 3)<br>+ + (Aug. 9) |
| No. 8<br>2 m.<br>male | + | CF <2 (May 4)<br><2 (June 3)<br><4 (July 2)<br>NT – (May 4)<br>– (July 2) |
| No. 9<br>2 m.<br>male | – | CF <2 (May 16)<br><2 (June 3)<br>4 (July 2)<br>NT – (May 16)<br>– (June 3) |
| No. 10<br>1 m.<br>female | – | CF <2 (May 4)<br><2 (June 3)<br>4 (July ?)<br>NT + (May 4)<br>+ (July ?) |
| No. 11<br>1 m.<br>female | – | CF (May 4)<br>< (June 3)<br>4 (July 2)<br>NT ± (May 4)<br>– (July ?) |
| No. 12<br>1 m.<br>female | – | CF 16 (May 4)<br>8 (June 3)<br>4 (July 7)<br>NT – (June 3)<br>– (July 7) |
| No. 13<br>2 m.<br>male | + | CF 2 (May 4)<br><2 (June 3)<br><4 (July 2)<br>NT – (May 4)<br>– (July 2) |
| No. 14<br>1 m.<br>female | – | CF 4 (May 16)<br>2 (June 3)<br>4 (July 2)<br>NT – (May 16)<br>– (July ?) |

molysin (APn). References for these reactions are given elsewhere [19]. A more than two-fold rise in antilysin reactions was considered significant.

## Results

*Serological findings.* Adenovirus type 5 was the only virus recovered. As shown in Table 1 it was found in 7 out of 14 infants. All virus strains were recovered in HeLa cultures only. The first virus strains were obtained on May 4 from four infants, all living in room No. 1. Two other infants (case 5 room No. 1 and case 13 room No. 3) excreted the same type of virus strains in stools on May 17 and 25

The incubation period seemed to be 7–9 days, which is in line with previous reports [1., 13].

## Discussion

In room No 1 fresh infection with adenovirus type 5 was obviously well correlated in time with the occurrence of febrile pharyngitis. With one exception no serological responses to any other aetiological factor were found, the causative role of adenovirus seems probable In cases 8 and 13 in room No 3 however the association of excretion of type 5 with respiratory illness seems more questionable as in these very young infants, no serological response occurred against the virus isolated.

The results of our virological study are in line with a newly published report by Vargosko *et al.* [23]. In a large survey from 1957–1963 they found that adenovirus excretion in older infants was associated with a higher percentage of CF antibodies against adenovirus than in younger infants in whom the excretion of adenovirus occurred more often without serological response

In only one infant serological response to a bacterial infection was demonstrated. This case had a mixed infection with adenovirus type 5 and staphylococci. The pneumococcal infection, which occurred in one of the sick staff members, was obviously not spread to the infants

During an outbreak of RS virus infection in 1964 in the same home the attack rate was 100%. During the present outbreak of adenovirus infection the attack rate varied from very high among the older infant in room No. 1 to fairly low among the younger infants in the other rooms. This difference in attack rates may indicate that an intimate type of exposure to adenovirus type 5 is necessary for the spread of this type of infection from person to person The contact between the infants in room No. 1 where the attack rate was nearly 100%, was indeed very close because the infants were placed together on the floor In rooms No 2 and 3 the infants were kept in their beds. The significance of maternal immunity in these small infants is questionable [3 1 ] Neutralizing antibodies against adenovirus type 5 were found in only two of the seven infants in rooms No 2 and No 3

According to our study in 1964 RS virus infections [18] were associated with acute respiratory illness in all cases In addition one-third had severe lower respiratory symptoms, i e bronchiolitis and bronchopneumonia. The adenovirus type 5 infection studied here was associated with upper respiratory illness (pharyngitis) only

## Summary

An outbreak of febrile pharyngitis occurred in May 1965 in a home for infants in Stockholm, Sweden Nine out of 14 infants, 1–10 months old, and 4 out of 1 staff members fell ill with fever (≥37.4 C) and upper respiratory illness. By the use of virological and bacteriological methods including attempts at isolation of virus and bacteria as well as serological tests against number of antigens (viruses, Mycoplasma pneumoniae and bacteria) a fresh adenovirus type 5 infection was demonstrated in 5 infants 4–10 months old living in the same room Another infant in this room excreted adenovirus type 5 but no serological study was carried out

in this case. Two other very young infants living in another room excreted type 5 virus, too, but in these cases serological evidence of fresh adenovirus infection was lacking. It was possible to rule out infection with influenza A and B, parainfluenza virus types 1, 2 and 3, mumps virus, psittacosis virus, RS virus, Mycoplasma pneumoniae, pneumococci and β-streptococci. A mixed infection with staphylococci and adenovirus type 5 was observed in one infant. No staff members excreted adenovirus in their stools. A fresh pneumococcal infection was demonstrated in one of the sick staff members.

Proven fresh adenovirus infections were associated with febrile pharyngitis. It seems probable that the virus found played a causative role in the occurrence of this outbreak.

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# Cardiac Output Estimation in the Newborn Infant

## *Modification of the Forward Triangle Factor*

*by* JOHN S. HANSON [1] WILLIAM OH,[2] RENÉ A. ARCILLA,[3] GÖRAN WALLGREN[4] and JOHN LIND

In conjunction with the widespread increase of investigations defining various parameters in the neonatal period indicator dilution studies have had their range of applications extended. Estimation of newborns cardiac output intra-cardiac and vascular shunts, circulation times and pulmonary blood volume are all adaptable to study by dye-dilution techniques

Because left to-right shunts occur so frequently in normal newborn infants as well as in those with cardiac malformations, cardiac output determinations from dilution curves in this age group have of necessity employed the so-called "forward triangle" method of calculation. This situation arises by virtue of shunt appearance soon after the curve's peak concentration has been attained, thus obviating a logarithmic re-plotting of points along the primary curve's exponential decay portion.

Implicit in application of the forward triangle method is employment of a factor which was derived by Hetzel *et al* [4] from dye curves recorded in adults. The original factor value suggested was 0.37 This was subsequently revised by another group [1] to 0.34 and quantitative studies employing this method of dye-curve analysis, whether in adults or infants, have since made use of one of these values.

During the early stages of a study to define normal hemodynamic relationships in the newborn human, it became apparent that a modification of the forward triangle constant might well be justified for this particular age group now being so actively studied. The present communication presents the results of the investigation whose purpose it was to clarify this important practical consideration.

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## Material and Methods

Six normal infants aged 22–54 hours (mean: 44.4 hours) were studied. Birth weights varied between 3300 and 3790 g and body surface area between 0.22 and 0.24 $m^2$.

Following sterile preparation of each infant's umbilical stump, two 5F plastic infant feeding tubes were inserted int the umbilical vein and an umbilical artery. These were then advanced until the venous catheter was positioned in the left trium and the arterial catheter in the proximal aorta. Analysis of several factors was used to establish catheter position: direct pressure recordings, pO determinations of blood samples, and comparison of the known length of catheter inserted with anatomically-determined measurements of vascular lengths. Continuous electrocardiographic monitoring was practiced, and all recordings f pressure were made with simultaneous registration of electrocardiogram and phonocardiogram on a direct writing instrument.

Detailed description of the instrumentation and methodology involved in dye-curve registration and calibration is presented elsewhere [3]. In brief, a Waters X-301 cuvett densitometer capable of background dye suppression was employed with a sterile cuvette and connecting t bing Cardio-green[2] dye of 0.5 mg/ml concentration was injected in 0.4 ml doses through the left atrial catheter by means of constant volume syringe Sampling via the proximal aortic catheter was accomplished at rate of 16–17 ml/min with a Sage-W ters constant-speed withdrawal-infusion pump fitted with a sterile 20-cc syringe Calibrations and inscription of dye curves were recorded on a direct writing recorder with paper width of 230 mm t a dri e speed f 4.23 mm/sec. Following completion of each dye curve all blood was reinfused to the infant and the system flushed with sterile heparinized saline solution. An absolute minimum of blood loss and of saline infusion was sought after in an attempt to maintain blood volume homeostasis and not introduce hemodynamic alteration artifacts. I view of published reports 5] the estimated blood loss of 20–25 ml during a study comprising registration of 20 dye curves was insignificant in this regard.

A total of 60 large-amplitude (130–220 mm) dye curves without evidence of shunt was recorded in this group employing left atrial injection and proximal aortic sampling The absence of shunt was ttested tonot only by the very contour of the curves themselves but through lack of any dye ppearance prior to re-circulation after proximal aortic injection and left atrial sampling A minimum of 4 points on each curve downslope maintained a linear relationship when plotted logarithmically and as many as 7 points could be so demonstrated in some curves.

For evaluation of the forward triangle area relative to total curve area, the classic method of Hamilton [2] for curve quantitation was used. The forward triangle method involves measurement of the time from curve onset to peak concentration (build up time, BT) and of peak concentration (PC) itself These factors and the amount of dye injected (I) are related according to the equation:

$$\text{Cardiac output} = \frac{60\ I}{(0.5)\ (BT)\ (PC)}\quad K.$$

A typical curve derived in this study and calculation of cardiac output from it are presented in Fig 1

## Results

A mean value of $0.23 \pm 0.005$ S.E. for the forward triangle constant was obtained from curves subsequent to left atrial injection. The corresponding figure for right atrial injection was 0 20 Table 1 presents a summary of these results and calculated standard errors. Frequency distribution of the values for K encountered in the 60 curves is presented in Fig 2.

The factor of 0.23 represents a 33 % red ction of the 0.34 constant previously suggested for adults and applied in some

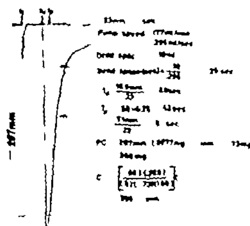


Fig. 1. ormal dy curve calculations. $AT_u$, uncorrected appearance time; $AT_c$, corrected appearance time; $BT$, build-up time; $PC$, peak concentration; $I$, amount of injectate; $C.O.$, cardiac output; $t$, injection time; $t_a$, appearance time; $t_p$, peak concentration time.

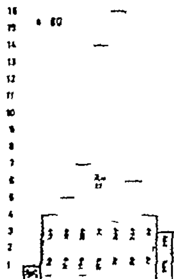


Fig. — Frequency distribution of forward triangle factor $K$ calculated from d curves with left trial injection and proximal aortic sampling in normal newborn infants.

studies involving infants in the neonatal period. When the 00 left-atrial dye curves were re-calculated using the new factor instead of 0.34 the mean difference between cardiac output calculated by the Hamilton and forward triangle methods decreased from −0.3‾ l/min to −0.030 l/min. Graphic representation of these difference obtained after employment of both constant values is presented in Fig. 3.

## Discussion

The recent interest accorded the human organism's physiological adaptation to extra uterine life has far reaching consequences. Studies centered on the neonatal period are of great importance not only for the establishment of "normal values" but as a basis for altering previous perhaps fallacious concepts studying disease states and devising therapeutic programs. Examples of such applications may be found in the question of late versus early clamping of the umbilical cord and study of the respiratory distress syndrome. It is therefore essential that investigations conducted in this particular population embody the basic tenet that methods and theories applicable to studies of older children and adults may require considerable revision. The newborn infant can by no means be



Fig. 3. Frequency distribution of differences in cardiac output calculated by Hamilton re-plot method and forward triangle method using factors for the latter of 0.34 and 0.21.

considered a "micro" version of the adult and consequently mere miniaturization or direct transference of study techniques valid for the adult may not pertain.

The major developmental work which produced a reliable method for dye curve analysis by the forward triangle method was conducted in adults [1 4] In addition, many of the patients whose curves were analyzed had acquired or congenital heart disease In both studies mentioned the vast majority of dye curves was recorded after peripheral dye injection and/or peripheral sampling That the integral forward triangle constant thus derived is not valid for dye studies in infants where central injection and sampling are performed is therefore understandable

As previously mentioned, important data can be and has been obtained in newborn infants through adaptation of accepted indicator-dilution techniques. Quantitative evaluation of dye curves more often than not requires employment of the forward triangle formula, and even in the absence of primary curve distortion by shunt this method of calculation is much simpler than that of Hamilton The derived values for cardiac output so obtained and the associated range of variability are generally considered to validate usage of the simplified method. It is hoped that the present modification will increase this reliability in the neonatal circulatory studies which are so necessary

## Summary

Indicator-dilution studies were performed in 6 infants 22–24 hours of age to examine the applicability of the commonly accepted forward triangle constant of 0.34 to cardiac output estimation in this age group From a total of 60 curves which were recorded following left atrial injection and proximal aortic sampling and which were without evidence of superimposed shunt, the area of the forward triangle was found to constitute 0.23 of the total primary curve area. Re-calculation of the 60 curves with this factor greatly improved the correlation of cardiac output values with those derived by the Hamilton method as compared to the correlation obtained with the 0.34 constant The significance of this modification for neonatal hemodynamic studies is discussed

## Acknowledgment

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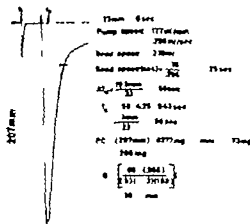


Fig. 1. Normal dye curve calculations. $AT_u$, uncorrected appearance time; $AT_c$, corrected appearance time; $BT$, build-up time; $PC$, peak concentration; $I$, amount of injectate; $C.O.$, cardiac output; $t_i$, injection time; $t_a$, appearance time; $t_p$, peak concentration time.

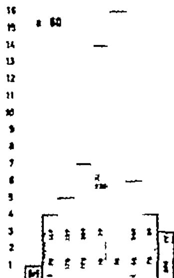


Fig. 2. Frequency distribution of forward triangle factor $K$ calculated from dye curves with left atrial injection and proximal aortic sampling in normal newborn infants.

studies involving infants in the neonatal period. When the 60 left-atrial dye curves were re-calculated using the new factor instead of 0.34, the mean difference between cardiac output calculated by the Hamilton and forward triangle methods decreased from −0.2** l/min to −0.030 l/min. Graphic representation of these differences obtained after employment of both constant values is presented in Fig. 3.

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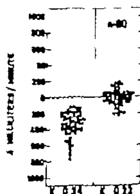


Fig. 3. Frequency distribution of differences in cardiac output calculated by Hamilton re-plot method and forward triangle method using factors for the latter of 0.34 and 0.23.

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# The Ecology of Infant Weight Gain in a Pre industrial Society

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Evidence deriving from several pre-industrial societies indicates that weight gains made by children during the first three months of life are generally at or above the average for infants in advanced European or American countries [1 6 8, 10 11 13 15] Thereafter a deceleration in weight gain and in overall growth occurs and is accompanied by severalfold increments in mortality [7 12]

The precise causes for this growth pattern are only partially understood and a more complete understanding awaits detailed ecologically oriented longitudinal studies of growth in representative communities. A beginning has been made in the detailed inquiry by McGregor *et al* [5] and provides a valuable supplement to hospital based studies such as that of Janardan & Suddhendu [4] However pre-industrial communities are by no means homogeneous either in their social structures or in the specific factors which in each are associated with deficient growth and development in infants. The present paper seeks to extend our understanding of the factors contributing to such maldevelopment by reporting the results of an ecological longitudinal study of weight gains made during the first six months of life by all children born in a representative Central American village in the course of a nineteen month period

This work was supported in part by grants from the Association for the Aid of Crippled Children, New York, U.S.A., the Nutrition Foundation, Inc the Milbank Memorial Fund, and the William Waterman Fund for the Combat of Dietary Diseases. The collection of the material was done during the time Joaquin Cravioto, M.D. was commissioned by the Panamerican Health Organization, Regional Office of the World Health Organization (WHO) as Associate Director of the Institute of Nutrition of Central America and Panama (INCAP)

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## The Setting

The investigation was carried out in the village of Santa Catarina Barahona, a community of approximately 1000 inhabitants located in the central Guatemalan plateau with the following characteristics:

### *Description of the village*

All the infants studied belonged to families living in Santa Catarina Barahona. This community part of the Cakchiquel linguistic group, is located in the Department of

Sacatepéquez in the central zone of the Republic of Guatemala, C.A. It lies at an altitude of 4 40 feet above sea level. The climate is characterized by two well-defined seasons—one dry and one with heavy rains which in popular usage are respectively referred to as "summer" and "winter."

The population was composed of 22 families making up a total of 896 persons, 149 of whom are below 5 years of age.

The crude mortality rate 2..3 per thousand in 1948 has diminished progressively to reach the figure of 9 9 in 196.. The age-specific mortality rate for children below 5 years which was 19 9 in 1948 was 6 6 in 196.. The birth rate has shown marked fluctuations during the last 15 years with a maximum of 5..6 in 1943 and a minimum of 33 1 in 196.. ( ).

*Production and commerce*

The villagers of Santa Catarina are small farmers whose main crops are corn, beans, chicken-peas, some greens such as lettuce and cabbages, and some fruit such as oranges and plums. There is commercial interchange between the village and both the capital city of the country and the main town in the neighboring Department of Escuintla.

*Migration*

Approximately 70 per cent of the heads of the families make a transient emigration four times during the year to the coastal region, and during the time of the coffee harvest about .3 per cent of the families travel daily between the village and the larger farms where both man and wife work as wage earners. Actual emigration, i.e. leaving the village permanently is so rare that it can be confidently stated that for practical purposes there is no emigration.

*Transportation*

Buses to and from the capital city are available six days a week. Transport to Escuintla and to the Pacific coast is available twice a week.

## Method and Procedure

The cohort of children studied consisted of all births occurring in the 10-month period beginning August 1 1963 and ending March 30 1964. In all 94 live infants and 4 still births were born in this period. Four children died during the first month of life and two more died between the first and third months of life. The remaining 8 survived for the whole period of study. Birth weights were obtained by the research staff in all cases within 48 hours after delivery. Each survivor was followed longitudinally for a six month period beginning with birth. Weighing was conducted during the follow-up period at intervals of one month plus or minus 3 days. All infants were weighed wearing minimal clothing. The clothing was then weighed separately and subtracted to obtain a true measure of unclothed weight. Weighing was carried out on a standard steel yard balance periodically calibrated against a series of standard weights.

At the time of weighing a full pediatric clinical examination was made on each infant. In addition to the health history taken at this time a home visit was made every fifteen days by a field worker to obtain an interim health and illness history.

A dietary history covering feeding during the previous month was also obtained on each weighing day. In addition, on that day a complete dietary record was made by the field worker who weighed, measured and recorded all supplemental foods offered to the child and ingested by him. Additional dietary information was obtained in the course of interim home visits.

Prior to the birth of the child, or as soon as was feasible after delivery in the remainder, a series of interviews was initiated by the field worker. These interviews were conducted in the course of several visits and were concerned with obtaining detailed and standardized information on family size and structure, parental occupation, income and expenditures, education, literacy and contact with mass media such as newspapers and radio. At the time of interview and repeatedly during the course of the interim

TABLE 1 *Pattern of feeding during the first six months of life in two groups of infants having different rates of weight gain*

| Months after birth | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| | | *Percentage of infants* | | | | | |
| Feeding on mother milk only | L | 50 | 0 | 0 | 0 | 0 | 0 |
| | U | 25 | 1_5 | 25 | 5 | 1_5 | 12.5 |
| Supplements added t mother's milk | L | 50 | 100 | 100 | 100 | 100 | 87.5 |
| | U | 75 | 87.5 | 75 | 75 | 87.5 | 87.5 |
| Other foods without mother milk | L | 0 | 0 | 0 | 0 | 0 | 12.5 |
| | U | 0 | 0 | 0 | 0 | 0 | 0 |

L – Infant with weight gain of less than 510 g/month
U – Infants with weight gain f more than 750 g/month.

visits estimates f sanitary cond tions and adequacy of the home as well as of the per sonal cleanliness and hygiene characteristic of each f the parent and of the child were made by the field in vestigator

The birth weights of the children studied were all between 1680 and 4750 g Fourteen of the 78 infants had birth weight of less than 2500 g with only 2 of these being under 2000 g Only 4 children had birth weights of 4000 g or more The mean birth weight was 3050 ± 560 g

As may be seen from Fig 1 verage weight gain for the cohort as a whole proceeded in a manner which was not different from that to be found in advanced communities during the first six months of life. However such an erage curve is deceptive since it obscures the fact that the population of infants is in fact bifurcating int identifiable groups during this period The first of these is gaining weight t a relatively high level whereas the second shows a lesser weight gain and a difference in the slope as well as in the inflection of the incremental curve These differently defined groups are illustrated in the curves for weight gain shown in Fig. 2. In the present cohort of children 26 individuals fell into each group. In one f the groups average monthly weight gain exceeded 750 g for each individual. In the other no infant had an average monthl eight gain of more than 510 g These two groups were selected for a comparati analysis of factors affecting weight gain in infants in pre-industrial community

## Results

Three sets of factors have in general appeared to be in various degrees associated with weight gain during infancy The first of these is the availability of nutrients to the child and involves both the amounts and the quality of the foods taken. The second, involves features of health not directly associated with intake which may indirectly affect nutritional status and so weight gain. Most promi nent mong these factors are infectious disease and parasitic infestation. The third set of influences are of more general social character and include such things as f mily circumstances social and economic status, educational background and patterns of child care Each of these sets of variables will be considered in turn

### *Nutritional practices*

The relation of the pattern of feeding during the first six months of life in the

TABLE 2. *Consumption of calories from sources other than human milk in two groups of infants receiving supplemental food during the first six months of life*

| Intake of calories/kg/day | Group | % of children at each month of life | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| < 20 | L | 75 % | 25 % | 43 % | 12.50 % | 14 % | 14 % |
| | U | 75 | 83 | 67 | 20 | 20 | 0 |
| 20–39 | L | 25 | 62.5 | 14 | 0 | 0 | 0 |
| | U | 25 | 0 | 17 | 40 | 0 | 0 |
| > 40 | L | 0 | 12.5 | 43 | 87.50 | 86 | 86 |
| | U | 0 | 17 | 17 | 40 | 80 | 100 |

L = Weight gain 510 g/month
U = Weight gain 750 g/month.

two groups of infants exhibiting different courses of weight gain are summarized in Tables 1, 2, and 3.

As may be seen from Table 1, little difference existed between the two groups in terms of whether the child was fed solely on mother's milk or had supplements added to such feeding during the first six months of life. Only in the first month was there any high degree of supplementation favoring the upper weight gain group. Thereafter supplementation appeared to be equivalent until the sixth month at which time one further factor of difference was manifest. At this age 12.5 per cent of the lower weight gain children were no longer receiving any mother's milk and were subsisting entirely on extra maternal food sources.

The character of supplementation, when it occurred, is considered in greater detail in Tables 2 and 3. In Table 2 the data on the ingestion of supplementary calories, independently of their nutritional quality

TABLE 3. *Amount of protein from sources other than human milk consumed during the first six months of life in the two weight gain groups*

| Protein intake g/kg/day | Group | Children of each month after birth | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 0– | L | 75.0 % | 0 % | 17 % | 0 % | 0 % | 17 |
| | U | 100 | 40 | 28 | 43 | 16 | 20 |
| < 1 g | L | 12.5 | 60 | 67 | 0 | 0 | 33 |
| | U | 0 | 60 | 62 | 57 | 84 | 80 |
| 1–1.99 | L | 12.5 | 20 | 0 | 80 | 50 | 0 |
| | U | 0 | 0 | 0 | 0 | 0 | 0 |
| > 2 | L | 0 | 20 | 16 | 20 | 50 | 50 |
| | U | 0 | 0 | 0 | 0 | 0 | 0 |

L = Weight gain 510 g/month.
U = Weight gain 790 g/month.

is summarized. In Table 3 the quality of the supplemental diet is considered from the point of view of protein intake.

As would have been expected from the overall lack of difference in the pattern of supplementation noted previously the ingestion of calories per kilogram of body weight per day did not differ significantly in the two groups of supplemented infants. A slight trend of difference favoring the low weight-gain group can be noted (Table 2) if intake of more than 40 calories per kilo per day is analyzed. This trend was most marked during the third and fourth months of life Before this age few or no children received so high a level of supplementation and after it almost all supplemented infants received enough supplemental food to place them in this group.

As may be seen from Table 3 the supplemental protein intake of low weight gain infants was higher t all ages. In contrast to the high weight gain infants, none of whom received supplemental protein during the first month of life 25 per cent of the low weight gain infants were given

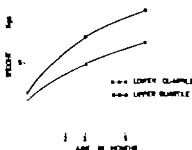


Fig. 2

such supplements at this time The general trend of difference continued through the remaining five months of life, and was highlighted by the fact that whereas at no age did any high weight infant receive 2 or more grams of protein per kilo per day of additional protein, such levels of supplementation were achieved by 50 per cent of the low weight gain infants by the sixth month of life

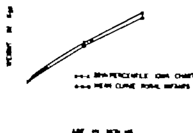


Fig 1

*Illness*

The most prominent illnesses during th age period studied were diarrhea and upper respiratory infection. A scattering of der matitides and one case of severe conjunc tivitis also occurred Although sporadic cases of measles, chicken pox, and other common diseases occurred in children in the village no cases of these disorders were diagnosed or suspected in the cohort of infants under six months of age. Patterns of morbidity resulting from illnesses in the two weight gain groups re presented in Tables 4 and 5

TABLE 4. *Pattern of morbidity during the first six months of life in two groups of infants differing in rate of weight gain*

| | Months after birth | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | % of infants ill for 15 or more days of the month | | | | | |
| Group with a rate of less than 510 g/month | 0 | 16 | 24 | 16 | 0 | 2 |
| Group with a rate of more than 750 g/month | 0 | 32 | 0 | 8 | 24 | 12 |

Significant illness for the purpose of analysing morbidity pattern was considered as cumulative sickness encompassing 15 or more days of any month of life (Table 4) Although differences in the age specific frequency with which such illness occurred was noted between the two groups, no significant difference existed between them in the frequency with which such prolonged bouts of sickness occurred over the whole 6 month period of observa

TABLE 5 *Morbidity due to diarrhea and/or upper respiratory infections in two groups of infants during the first six months of life*

| % of life being sick | All illnesses L | All illnesses U | Diarrhea L | Diarrhea U | Upper respiratory infection L | Upper respiratory infection U |
|---|---|---|---|---|---|---|
| | % of infants suffering from | | | | | |
| 0 | 22 | 17 | 38 | 34 | 31 | 41 |
| 1 19 | 36 | 75 | 46 | 66 | 44 | 50 |
| 20–39 | 23 | 8 | 8 | 0 | 15 | 8 |
| 40–60 | 16 | 0 | 8 | 0 | 8 | 0 |

L % of infants from group with body weight gain of less than 510 g/month.
U % of infants with body weight gain of more than 750 g/month.

tion. A somewhat different picture emerged when the data on total duration of identifiable illnesses, independently of the duration of any specific illness, were analyzed (Table 5) When the proportion of time spent in being sick during the first six months of life was compared in the two groups it was found that a significantly greater proportion of the low weight gain infants had cumulated illnesses totaling 20 or more per cent of their lives ($\chi^2 = 6.92$, $p < 0.01$) Further whereas 16 per cent of the low weight gain infants had cumulated illness time *equal to or greater than 40* per cent of the life span, no individual in the upper weight gain group had cumulated illnesses of this duration.

When diarrhea and upper respiratory infections are separately considered a similar trend was manifested. Both of these types of illnesses tend to be more frequently present for cumulatively longer periods of time in the lower weight gain group However the obtained differences did not reach acceptable levels of statistical significance

## *Family and social background*

In considering family and social background factors associated with weight gain as well as aspects of child care analysis was restricted to those characteristics on which the available observational and interview data permitted us to have detailed and accurate information on the families of all the children studied. The following variables could be characterized in this way 1) family size 2) parental age, 3) total annual family income per capita, 4) total annual family expenditures per capita 5) percentage of total annual in-

come spent on food, 6) percentage of total expenditures devoted to food, 7) per centage of total expenditures for health services 8) home conditions and environ mental sanitation, 9) personal hygiene of parents and infants 10) literacy of parents, 11) contact with mass media 12) language usage when talking to children. The low and high weight gain groups were com pared for each of these variables

I *Family size* The differences which characterize the size of the families from which the low and high weight gain infants derived re summarized in Table 6 Num ber of family members in this table refers only to the nuclear family of parents and siblings. It is clear from the table that the upper weight gain children derived from smaller families than did those infants showing low weight gains. When number of children in each group deriving from families having less than 5 versus 5 or more members was considered the relia bility of the hypothesis was confirmed ($\chi^2 =$ ~~, $p < 0.01$) When extended family size was considered (with nuclear family size excluded) no difference in number of living relatives present in the village wa found.

TABLE 6. *Family size two groups of in fa ts differing a rate of weight ga n*

| Number of members | Percentage of families with L | U |
|---|---|---|
| 3–4 | 38 | 75 |
| 5–6 | 24 | 25 |
| 7–8 | 38 | 0 |

L Infant with body weight increments of less than 510 g/month.
U Infants with bod weight increment f more than 750 g/month.

TABLE *Parental age of two groups of fants differing in rate of weight ga n during the first six months of l fe*

| Age of | of parents of infants with body weight increments of Less than 510 g/month | More than 750 g/month |
|---|---|---|
| Mothers | | |
| 17–30 yrs. | 38 | 5 |
| 30–44 | 62 | 25 |
| Fathers | | |
| 20–30 yrs. | 25 | 33 |
| 30–40 | 58 | 67 |
| 40–50 | 17 | 0 |

_ *Parental age* The data on age of the parent in the two groups of children are summarized in Table There was a clear difference in mother age between the two groups of infants. The mothers of the high weight gain group were significantly younger ($\chi^2 =$ ~ _, $p < 0.01$) Father s age also tended to differ in th same direction, with 1 per cent of the fathers of the children in the low weight gain group and none of the fathers of the high weight gain children over 40 years of ge These paternal age differences, however did not reach an acceptable level of statistical significance

3 *Family incom* In all but three cases the children came from peasant families The exceptions involved were one child in each group whose parents were govern ment clerical workers, and one child in the upper weight gain group whose f ther was a skilled craftsman. In the main, the head of families were small proprietors or renters of small farms. In some cases income was ugmented by agricultural work on large scale farms during special

TABLE 8. *Income and types of expenditure in families with infants significantly different in rates of weight gain*

| | | % of families with infants with monthly average increments of | |
|---|---|---|---|
| | | Less than 510 g | More than 750 g |
| Annual total income per capita in U.S.A. $ | > 90 | 0 | 33 |
| | 60–89.9 | 31 | 17 |
| | 30–59.9 | 43 | 50 |
| | 20–29.9 | [illegible] | 0 |
| Total expenditure per capita per annum in U.S.A. $ | 90–120 | 0 | [illegible] |
| | 60–89.9 | 22 | 25 |
| | 30–49.9 | 69 | 58 |
| | 20–29.9 | [illegible] | [illegible] |
| % of total income spent on food | 10–29.9 | 0 | 17 |
| | 60–89.9 | 46 | 25 |
| % of total expenditure spent on food | 60–79 | 62 | 42 |
| | 40–59.9 | 38 | 58 |
| % of total expenditure paid for health services | 0 | 8 | 5 |
| | 0.15–4.99 | 62 | 83 |
| | 5–10 | 30 | 9 |

Total income defined as cash income plus cash value of income other than on a cash basis, e.g. produce housing services etc.

seasons, or by migratory work during the coffee harvest.

Economic data for the families of both groups of infants are summarized in Table 8 and Fig. 3. Clearly the higher weight gain children tended to derive from families having a higher per capita annual income. Thirty three per cent of the families in this group had an annual total per capita income of 90 U.S. dollars or more. No family in the lower gain group was at this economic level. Further at the lower end of the income scale 70 per cent of the low weight gain families in contrast to 50 per cent of the high weight gain ones had total annual per capita incomes of less than 60 dollars.

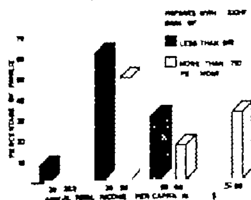


Fig. 3

Total expenditures were slightly higher in the families of the upper weight gain group. Moreover the distribution of expenditures tended to be significantly different. The families of the high weight gain infants spent a lower proportion of total income on food than did the families of the low weight gain children. Whereas 46 per cent of the low weight gain families spent between 60 and 90 per cent of total income on food only 25 per cent of high

TABLE 9 *Environmental sanitation and personal hygiene in families with infants significantly different in rates of weight gain*

| | | % of families or individuals | |
|---|---|---|---|
| | | Infants with weight gain of less than 510 g/month | Infants with weight gain of more than 750 g/month |
| Environmental sanitation (Home conditions) | <10 | 50 | 25 |
| | 10–15 | 25 | 42 |
| Sanitary score | >15 | 25 | 33 |
| | Hygiene score | | |
| Personal hygiene Fathers | 30–50 | 59 | 18 |
| | 50–70 | 33 | 64 |
| | 70 and above | 8 | 18 |
| Mothers | 30–50 | 38 | 25 |
| | 50–70 | 46 | 67 |
| | 70 and above | 16 | 8 |
| Infants six months old | 30–50 | 23 | 17 |
| | 50–70 | 61 | 83 |
| | 70 and above | 16 | 0 |

weight gain families did so Further none of the low weight gain families spent less than 30 per cent of total income on food, whereas 17 per cent of the high weight gain families achieved this low level of relative expenditure The trends are more sharply delineated when one considers the percentage of total expenditures rather than of total income devoted to food in the two groups of families. As may be seen, the high weight gain families devoted a significantly smaller proportion of their expenditures to food ($\chi^2 = 3.89$ $p < 0.05$)

In view of the differential illness history in the two groups of infants it was anticipated that a greater proportion of total expenditures would be devoted to payment for health services in the families of the low weight gain group This expectation was confirmed

4 *Home conditions and environmental sanitation* Home conditions and environmental sanitation were scaled com posite estimate deriving from the multiple systematic observations on home conditions and environmental cleanliness made by the field worker The data were accommodated to a scale the maximum score on which was one hundred, a value which would be reached in a well-constructed, well kept middle-class home In the village no household chieved a summated score of more than 30 The differences in environmental sanitation and home conditions estimated in this manner are compared for the two weight gain groups in Table 9 A tendency existed for the low weight gain children to come from households having low level of environmental sanitation Fifty per cent of the households for this group of infants were scored at 0 or less a value which reflected the absence of toilet facilities and separate washing places for clothes persons, food and food related objects, cohabitation of domestic animals and people cooking and

TABLE 8 *Income and types of expenditure in families with infants significantly different in rates of weight gain*

| | | % of families with infants with monthly average increments of | |
|---|---|---|---|
| | | Less than 510 g | More than 750 g |
| Annual total income[a] per capita in U.S.A. $ | >90 | 0 | 33 |
| | 60–89.9 | 31 | 17 |
| | 30–59.9 | 62 | 50 |
| | 20–29.9 | 8 | 0 |
| Total expenditure per capita/per annum in U.S.A. $ | 90–120 | 0 | 8 |
| | 60–89.9 | 22 | 25 |
| | 30–59.9 | 69 | 58 |
| | 20–29.9 | 8 | 8 |
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| % of total expenditure paid for health services | 0 | 8 | 8 |
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| | 5–10 | 30 | 9 |

[a] Total income defined as cash income plus cash value of income other than on a cash basis, e.g. produce, housing services etc.

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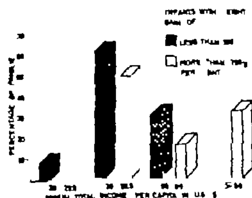


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INTERRELATION AMONG BIOSOCIAL FACTORS AND LOW WEIGHT GAIN

Fig 4

that 54 per cent of them were literate. Further although 83 per cent of the low weight gain group s fathers were literate only 4_ per cent read newspapers, a figure in marked contrast to the 82 per cent of newspaper readers found among the fathers of the high weight gain infants.

8 *Language usage with children* Since the language a parent in a pre-industrial culture uses in talking to a child reflects the degree to which identification with a more expanded sphere of social relations and a higher level of technology has been chieved, detailed information was obtained on language practices in the parents of the two groups of infants. As may be seen from T ble 10 eighty five per cent of the parents of low weight gain infants used only the native Indian dialect when speaking to a child, whereas only 15 per cent used Spanish in addition to the local dialect. In contrast, 4_ per cent of the parents of the high weight gain infants used Spanish as well as the native Indian dialect when talking to the child. The difference in language usage in the two groups is statistically significant ($\chi^2 = 4.96$ $p < 0.05$)

## Discussion

The findings of our study have indicated clearly that in an ethnically homogenous group living in a relatively undifferentia ted pre-industrial peasant community differences in patterns of infant weight gain are associated with a variety of biological and social variables. Certain of these variables have directions of associa tion with infant weight gain which were unanticipated. On the basis of previous

reports it would have been expected that low weight gain would have been associated with low levels of extra maternal food supplementation. Instead in children under six months of age poorest weight gain tended to be associated not with the lowest levels of supplementation but rather with levels of supplementation of food intake that were equal to or greater than those found in the highest weight gain group It is possible that this direction of association is related to the age of the children studied since most considerations of supplemental extra-maternal feeding have been concerned with older children who were weaned or whose maternal milk supply was failing If this is the case then failure to gain in weight must be viewed not as a general and uniform phenomenon but rather as one which has different age specific associations.

Of particular interest in view of the widespread concern with protein-calorie malnutrition is the finding that at least during the first six months of life higher levels of extra maternal calorie and protein ingestion were not associated with higher rates of growth. This lack of association, for the age group studied, may be based upon any one of a number of interrelated factors, at least four of which are both worth considering and readily capable of being explored.

It is possible that although intake is higher utilization of protein is either limited or reduced in those children who have low levels of weight increment. Such limitation may be intrinsic or may be based upon concurrent infectious illness Inadequacies of utilization could readily be investigated by means of systematically planned balance studies conducted on an appropriate sample of children during health and infectious illness. A partial model for such studies can be drawn from the investigations of Bressani, Wilson & Scrimshaw [14]

A second hypothesis to be considered is that high levels of supplemental calories and protein may be systematically related to failure of maternal milk supply or inadequacy in its ingestion. They may thus be associated with diminution of maternal milk supply with excessively early total or partial weaning or with interruptions of the normal nursing pattern. A number of social factors such as increased demands on the mother for work outside the home, demands for sexual availability when such behavior is traditionally proscribed during nursing or an early subsequent pregnancy all may result in a diminution of the amounts of maternal milk produced and ingested. If this is the case then extramaternal food sources represent substitute rather than supplemental feeding in the low weight gain group

A third possibility is that protein intake at levels above those already provided by mother's milk represent unutilized, and therefore unnecessary dietary supplements It is perhaps more important for infants who are being breast fed to receive supplementation in the form of other substances such as iron and vitamin C than for them to receive protein supplementation during this age period. Valuable information could be obtained by focusing on these aspects of infant nutrition in preindustrial communities.

The findings of the present study support the conclusion that low weight gain during the first six months of life is strongly

associated with the duration and frequency of infectious disease. Infants who have such disease during a considerable portion of early life are most heavily represented in low weight gain group However whether this pattern of infection is the cause of low weight gain, the consequence of increased vulnerability to infection because of poor growth or the product of modified individual inability to utilize ingested food as in diarrhea, cannot be answered by the present body of data. Further we cannot at present determine whether the low weight gain is a result of directly produced increases in catabolism during infection or the product of diminished food intake deriving directly from anorexia associated with infectious illness or indirectly from social customs, traditions and practices of reduced feeding frequently used in the primitive management of disease Answers to these questions can be obtained only by detailed individual case analysis of the time relations between infections and weight gain, by balance studies, such as those already suggested, and by detailed investigation of social practices in the treatment of sick infants. Certain of these studies are currently in progress.

The strongest set of relations found in our data and in our view those which most probably underlie the phenomenon of low weight gain during the first months of life in a pre-industrial culture, are those related to th general familial social and cultural milieu in which the child is developing These associations indicate that a familial complex which includes large family size older age and higher parity in the parents low income a larger proportion of expenditures devoted to food procurement, low levels of economic excess and reserves, low levels of education reflected in illiteracy minimal contact with contemporary knowledge through mass media of communication and a strong attachment to the traditional way of life as reflected in the exclusive utilization of the native dialect, are all strongly associated with low weight gain. A flow diagram suggesting several hypothetical pathways through which this complex of interrelations may result in low weight gains is presented in Fig 4

Starting with a low level of technology which results in limited income and the expenditure of excessive energy for the procurement of the bare necessities of life one is confronted with reduced purchasing power and with the absence of reserves and surpluses. At least two pathways can derive from this point to produce low weight gain in infants. The first is direct, and proceeds from limited resources to defective environmental sanitation, to the persistence of primitive conceptions of health, to unbalanced food distribution and utilization, to reduced ingestion by the infant and so to low weight gain. Indirect branching may occur when primitive conceptions of health [3 9] result in insufficient sensitivity to the hygienic requirements of the child, and lead to its exposure to infection which either directly or indirectly may produce low weight gain.

A second major pathway proceeds from lack of reserves and surpluses to pressures for early school leaving, high levels of illiteracy diminished opportunities for obtaining adequate information, persistence of primitive concepts of health and so back to pathway one Alternatively

early school leaving can result in an increased probability of marrying an equally uneducated spouse with consequent multiplication of opportunities for inadequate care of the child, illnesses and malnutrition, and so increased morbidity mortality and increased likelihood of low weight gain

Clearly many other cycles may be identified. However all of them result in a reinforcement of opportunities for poor growth, and for the persistence of a social way of life which is technologically deficient. Viewed ecologically low weight gain in infancy appears to be one manifestation of a social, economic and cultural complex characterizing pre-industrial society the alteration of which is probably necessary for optimal growth and development of children.

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REVIEW ARTICLE

# Problems in the Diagnosis of Hereditary Galactosaemia

## *A Material of 14 Patients with Hereditary Galactosaemia and 7 Patients Presenting Particular Problems of Differential Diagnosis*

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The aim of the present study has been to give an account of the special problems which may arise in the diagnosis of hereditary galactosaemia and to emphasize the importance of enzymatic diagnosis. We have described the clinical pathological, therapeutic and genetic aspects in more detail in earlier papers [4 5 23 4, 25].

Hereditary galactosaemia is a classical example of an inborn error of metabolism. The recessive genetically-determined absence of the cellular enzyme galactose-1 phosphate-uridyl transferase (transferase) [1 17] leads to the intracellular accumulation of the galactose from the diet in the form of galactose-1 phosphat [7 20].

The normal metabolism of galactose is as follows:

(1) galactose + adenosine triphosphate → galactose 1 phosphate + adenosine diphosphate (enzyme: *galactokinase*)

Galactose-1 phosphate then combines with the nucleotide uridine diphosphate glucose with the release of glucose 1 phosphate:

(2) galactose-1 phosphate + uridine diphosphate glucose ⇌ glucose 1 phosphate + uridine diphosphate galactose (enzyme: *transferase*)

The galactose in the galactose-containing nucleotide formed in this way is converted to glucose through the reaction.

(3) uridine diphosphat galactose ⇌ uridine diphosphate glucose (enzyme: *epimerase*)

and the uridine diphosphate glucose can again react with galactose-1 phosphate (reaction (2)).

In this way the dietary galactose in the normal subject enters the glucose metabolism of the body via glucose-1 phosphate

In patients with hereditary galactosaemia reaction (*) is blocked, and the resultant accumulation of galactose-1 phosphate is directly or indirectly responsible for the symptoms and signs of the disorder

Patients with galactosaemia are generally normal t birth and the consequences of the enzyme defect do not become apparent until it becomes necessary

for the infant to metabolise the galactose from the milk. However in a few cases galactose 1 phosphate has been demonstrated in cord blood, which implies that galactose can be transferred from the mother to the foetus [20]

The disorder becomes manifest with the intake of milk during the first days of life [13] Regular features are dyspepsia with vomiting and diarrhoea, failure to thrive jaundice and hepatomegaly and possibly swinging temperature; there is often a high concentration of galactose in the blood and urine and there is also proteinuria and abnormal amino-aciduria. In severe cases dehydration and liver failure, with ascites, splenomegaly and haemorrhagic diathesis, follow If the disorder is not diagnosed at this stage it may prove fatal In some cases the signs and symptoms may diminish during the subsequent months—despite the continued intake of milk—but these patients always show evidence of irreversible organic damage, namely oligophrenia, cataract and cirrhosis of the liver

Treatment consists of a galactose-free diet [8, 14], if this is introduced sufficiently early and followed rigidly then as a rule the patient's condition will rapidly improve, and he will remain free of symptoms and develop normally It should be remembered that dietary galactose is not essential for the synthesis of the cerebrosides and other compounds containing galactose as uridine diphosphate glucose can be converted to uridine diphosphate galactose by means of epimerase (reaction (3)) which is the galactose-donor in the synthesis mentioned.

## Enzymatic Diagnostic Procedures

The demonstration of the absence of galactose-1 phosphate-uridyl transferase is the specific diagnostic evidence of hereditary galactosaemia. Two techniques are used in the measurement of transferase; the Kalckar Tolstrup semiquantitative technique, and Tolstrup's quantitative method [1 25]. The simpler *semiquantitative method* makes it possible to distinguish between individuals with absence of transferase activity (patients with hereditary galactosaemia) and those with demonstrable transferase activity (diagnosis of hereditary galactosaemia excluded) and this method is therefore especially suitable for diagnostic purposes. *Quantitative measurement of the enzyme* can naturally also be used in diagnosis but the method is more complicated; in the present study it was used as a supplement to the semiquantitative method in six patients, and alone in one patient (patient 14, Table 1). In this patient exchange transfusion had been carried out at the age of 3 days, and the transferase concentration was therefore followed at regular intervals—the enzyme concentration fell at the same rate as the transfused erythrocytes were presumably destroyed and in this way the probability of the diagnosis of hereditary galactosaemia could be rapidly established, whilst the diagnosis received final confirmation when the enzyme concentration fell to 0 [5]

*Principle.* Transferase catalyses reaction ( ) Haemolysed whole blood is added to a reaction mixture containing uridine diphosphate glucose and galactose-1 phosphate in tris buffer Normal blood will catalyse the conversion of uridine diphosphate glucose whereas blood from patients with hereditary galactosaemia is not capable of bringing about this conversion. At the end of the reaction period the amount of uridine diphosphate glucose remaining in the reaction mixture is measured by means of differential spectrophotometry with the aid of uridine diphosphate glucose dehydrogenase

## Material

The authors consider that they are aware of all the cases of hereditary galactosaemia which have been diagnosed in Denmark. At the end of 1964 there were 12 such patients, the first of whom had been reported by Mortensen & Sondergaard in 1955 [19]. Eleven of these 12 patients have been included in the present material together with three patients from Norway making a total of 14 patients with hereditary galactosaemia.

During the years 1960–64 blood samples from 49 patients who were suspected of suffering from hereditary galactosaemia were received in the Central Laboratory Glostrup Hospital, Copenhagen, the diagnosis was confirmed by the absence of transferase in eight of these cases. Seven of the remaining 41 cases, in which the diagnosis was excluded by the demonstration of transferase activity will be described in the following, for the illustration of the problems which may arise in diagnosis.

Tolstrup introduced the enzymatic diagnosis of hereditary galactosaemia into Denmark in 1960 [23]; six of the 14 patients with hereditary galactosaemia were born before that dat Enzyme measurements have been carried out on all the surviving patients, 11 in all.

### *Patients with hereditary galactosaemia*

As can be seen from Table 1 the symptoms correspond to those which have been described in the literature. All patients were normal at birth. Two of the patients (nos. 11 and 13) received a galactose-free diet from birth as they had elder siblings in whom the diagnosis of hereditary galactosaemia had been established; neither of them have ever had any abnormal symptoms although one (no. 11) has, as an unusual sign an isolated abnormal methioninuria.

The patients who had received milk all had enlargement of the liver and only a few had not exhibited one or more of the following symptoms nd signs: dyspepsia, jaundice reducing substances in the urine or proteinuria. It is noteworthy that patient no 3 was apparently ble to tolerat milk during the first months of lif without developing dyspepsia. Fehling test was negative in two of the patients (nos. 9 and 10), and this presumably led to a delay in diagnosis. Pathological values f prothrombin, alkaline phosphatase and serum transaminase were found in those patients in whom the levels were measured.

Only patients nos. 8 and 10 were examined for amino-aciduria whilst receiving a diet containing galactose and in both cases an abnormal amount of non-specific amino acids was found in the urine In those cases in which the excretion of amino acids was investigated whilst the patient was receiving a galactose-free diet the findings were generally normal; an exception to this rule was patient no 11 who, as mentioned, excreted considerable amounts of methionine in the urine despite his galactose-free diet. The brother of this patient (patient no. 10) exhibited another unusual sign—hydrocephalus. In so far as we have been able to ascertain this has not previously been described in hereditary galactosaemia [22]

Three of the patient died before the introduction of transferase measurements into Denmark. One f these patients (no. 2) was diagnosed clinically but died of intercurrent disease, and one patient (no. 12) was suspected of having hereditary galactosaemia, but treatment was instituted too lat Patient no 4 died without the diagnosis of hereditary galactosaemia having been suspected; the urine was not investigated for reducing sugars, but the clinical history and autopsy descriptions were compatible with the diagnosis, which was made in the patient brother

It was thus possible t carry out transferase measurements in 11 patients. In 10 cases the semiquantit tive technique was used, supplemented in six cases by the quantitative technique whilst in one case only the quantit tive technique was used. In none of the patients was any significant transferase activity found in haemolysed whole blood.

At follow-up vestigation, 6 months to 5

TABLE 1 *Signs and symptoms of 14 galactos*

| Patient number | 1 | | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Sex | Boy | Boy | Boy | Girl | Boy | Boy | Boy |
| Year of birth | 1954 | 1956 | 1957 | 1958 | 1959 | 1960 | 1961 |
| Age of diagnosis, mths. | 2 | 2 | 4 | Post mortem | ½ | 1/3 | 1½ |
| Galactosaemia in elder siblings | − | − | + (no. 2) | − | + (no. 4) | − | − |
| Galactosuria/Fehling | +/ | +/+ | +/+ | | +/+ | /+ | /+ |
| Glucosuria (Clinistix) | | | | − | − | − | − |
| Proteinuria | + | + | + | + | − | | + |
| Dyspepsia | + | + | − | + | + | + | + |
| Icterus | + | − | + | + | + | + | + |
| Hepatomegaly | + | + | + | + | + | + | + |
| Cataract | + | + | + | | − | − | + |
| Diet | Milk-free diet | Soya flour | Gruel, mashed vegetables, glucose, olive oil | Milk mixture | Soya flour Velactin | Velactin | Gruel, Idon, Velactin |
| Follow-up investigation | Cataract mental retardation | Died at age of 4 months | Cataract, mental retardation | Died at age of 1 mth | Normal | Normal | Normal |
| Age at follow up | 7 yrs | | 3 yrs | | 5 yrs | 4 yrs | 16 mths |
| Special comments | | Operated for ileus at age of 3½ mths | Milk dyspepsia in infancy | Not diagnosed in life | | | Cataract disappeared |
| Semiquantitative measurements of transferase (UDPG-consumption in reaction mixture) | −2.6 % | | −0.3 % | | 0 % | −0.5 % | −0.3 % |
| Quantitative measurement of transferase (μmol UDPG/hr/g Hb) | −1 7 | | | | | 0.2 | |

years after the diagnosis had been made, 7 of the patients were found to be normal. In 6 of these patients the diagnosis had been established before the end of the 4th week of life (nos. 5 6, 8, 11 13 14), whilst in the 7th patient (no. 7) the diagnosis was not made until the 6th week of life, and this patient had a transient cataract. The 4 surviving patients who were not normal (nos. 1, 3, 9 10) were all mentally retarded; 3 of them also had cataract, and one enlargement of the liver In none of these patients had the diagnosis been made before the end of the 8th week of life.

*nts with the results of enzymatic investigations*

| | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|
| | Girl | Boy | Boy | Girl | Boy | Girl |
| | 1963 | 1963 | 1964 | 1958 | 1962 | 1963 |
| | ½ | 9 | 0 | Post mortem | 0 | ½ |
| | – | – | + (no. 10) | – | + (no. 12) | – |
| | +/– → + | –/– | –/– | +/+ | –/– | +/+ |
| | – | – | – | – | – | – |
| | + | – | – | + | – | + |
| | + | + | – | + | – | + |
| | + | + | – | – | – | + |
| | + | + | – | + | – | + |
| | + | + | – | – | – | |
| flour tm | Milk-free diet | Milk free toddler diet | Velactin | Milk-free diet shortly before death | Velactin, Rice-flour water | Velactin |
| al | Mental retardation, hepatomegaly | Cataract, mental retardation | Normal (Methioninuria) | Died at age of 2 mths | Suffered from pneumonias, otherwise normal | Normal |
| | 6 mths | 2 yrs | 1 yr | | 5 mths | 3½ mths |
| ente- oh) on one- ert | Cataract disappeared. Fehling – at first investigation at age of 6 weeks | Pt. has hydrocephalus | Cord blood analysed. Never received galactose. Coli enteritis | Suspected of galactosaemia, diagnosis not definitely confirmed. Sister to no. 13 | Never received galactose | Exchange transfusion at age of 3 days |
| | +2 % | +2 | –1· | | –0.7 | |
| | | 0.2 | –0.9 | | –0.2 | –0.7 |

Of the 10 patients who were the first cases t present in the family 8 were diagnosed in lif and 4 were found t follow up t be normal.

Of the 4 patients who had elder siblings with the same disorder all survived, and 3 were found t follow up t be normal. The fourth patient (no. 3) who was not normal t follow up had not exhibited one of the usual signs—milk dyspepsia—and the case was not diagnosed until he was 4 months old.

This material comprises 4 girls and [illegible] boys. A similar xcess f males has [illegible] reported in other materials [13]. [illegible] this may

this patient was found to be normal although it is possible that the cataract will be observed again at a later examination. Two of the patients never exhibited any of the symptoms or signs of the disorder the diagnosis having been established by means of diagnostic enzyme tests immediately after birth both these patients had elder siblings who were known to suffer from hereditary galactosaemia *Thus if the diagnosis is established before* the end of the 4th week of life and effective therapy instituted, it is possible to prevent the late sequelae in a number of patients, although in some cases even earlier diagnosis may be necessary Late sequelae are regularly observed when the diagnosis is not established until after the 8th week of life.

Investigations into the frequency of carriers of the disorder (heterozygotes) would suggest that there are in fact a greater number of undiagnosed cases than *has previously been suspected. As judged* from the number of diagnosed cases the frequency of the disorder as calculated from the material presented here is about 1 per 75 000 births, as 12 patients were diagnosed in Denmark in the period 1952–64. In an English material *the in*cidence is given as 1 per 70 000 births [*1] If the disease frequency is 1 70 000 then the frequency of heterozygotes in the [illegible] should be of the order of [illegible]

[illegible] es have a reduced
[illegible] ion and can by this
[illegible] from the the nor
[illegible] high degree of
[illegible] tions with the
[illegible] ency of hetero-
[illegible] ulation have
[illegible] apparently

normal persons who were found to have transferase concentrations at the heterozygous level being calculated. Such investigations have been reported in one Danish [5] and two American materials [2, 19] These investigations unanimously suggest that the frequency of heterozygotes is of the order of 1 60 and that the disease frequency is considerably higher than would appear from the number of cases which are diagnosed. *It is possible* that the disease frequency of hereditary galactosaemia should be considered to be of the same order as that of another inborn error of metabolism, phenylketonuria, i e. 1 per 10 000 births [18]. However because of the number of unclarified genetic problems it is necessary to have reservations about the calculation of the disease frequency from the presumptive frequency of heterozygotes

All things taken into consideration, there can be no doubt that the diagnosis *of hereditary galactosaemia is made too* rarely and in a number of cases too late. Because of the very good possibility of treating these patients, there are good grounds for maintaining a sharp awareness of this disorder

*The procedure in the diagnosis of* hereditary galactosaemia follows these main lines

1) the clinical picture of the illness;
2) the secondary biochemical changes;
3) the primary biochemical defect (ab*sence of enzyme*)

*ad* 1) Every child who develops one or more of the following symptoms and signs during the neonatal period must be suspected on clinical grounds of suffering from hereditary galactosaemia. dyspepsia,

failure to thrive jaundice with evidence of liver disease and irregularly fluctuating temperature. The diagnosis must also be considered in older children with one or more of the following symptoms and signs: cataract cirrhosis of the liver and mental retardation.

*ad 2)*: Galactosuria and hypergalactosaemia are the most important of the secondary biochemical changes. If the urine of every infant admitted to hospital were to be examined by Fehling's or Benedict's test at least once, and in suspected cases on several occasions, this would be an advance in the diagnosis of hereditary galactosaemia. The results of these tests are also positive if the urine contains glucose, pentose, fructose lactose or homogentisic acid, and routine testing would therefore lead to the diagnosis of inborn errors of metabolism other than hereditary galactosaemia. Abnormal findings in the laboratory investigations mentioned above including findings of proteinuria and abnormal liver function tests, will strengthen the suspicion of the diagnosis.

It is necessary to bear in mind the fact that these secondary biochemical changes are the result of the absorption of galactose, and that they may be absent if the patient vomits excessively

*ad 3)*: The primary biochemical defect the absence of transferase, is specifically demonstrated by the diagnostic enzymatic procedures mentioned earlier These tests should be carried out in all patients in hom there is a suspicion of hereditary galactosaemia.

The primary biochemical defect is naturally unaffected by any galactose-free diet which may already have been instituted whilst in contrast the clinical and secondary biochemical changes often disappear rapidly when the patient receives such a diet As is apparent from the present material, the prognosis depends upon the rapid institution of therapy and any patient who is suspected of suffering from galactosaemia—whether this suspicion is aroused by the results of urine tests or by the general clinical picture—should immediately be placed on a galactose-free diet for a period of observation, during which the transferase activity should be investigated. More detailed investigation of the secondary biochemical changes, including any possible attempt to discover whether the reducing substance in the urine is galactose, is completely unnecessary for the diagnosis of hereditary galactosaemia, and should not be permitted to delay the institution of adequate therapy Diagnostic attempts to accentuate the clinical and biochemical changes by means of galactose tolerance tests, such as were used before the introduction of the measurements of transferase activity are obviously also unnecessary and should be avoided, as the patient's condition may deteriorate alarmingly during such tests.

In those cases in which the patient has received blood transfusions it is not possible to use the more simple semiquantitative technique for the measurements of transferase activity but the probability of the diagnosis can be demonstrated from a relatively early date by means of quantitative measurements of transferase If repeated estimations reveal a falling concentration of th enzyme. The complete absence of transferase cannot be demonstrated until the last of the transfused erythrocytes are eliminated, and this may

take up to three months from the date of transfusion.

The diagnostic procedure outlined above with the introduction of a galactose-free diet during an observation period, should lead to an improvement of the prognosis in many children In families in which galactosaemia is known to occur it is now possibl to determine the genetic prognosis with greater certainty than hitherto, be cause of the reliable method of identifying the carriers of hereditary galactosaemia [25] Siblings of patients with galactosaemia should be placed on a galactose-free diet immediately after birth and observed for the disorder as was carried out in two of the patients in the present material—these patients never received any food containing galactose which is the optimal mode of treating the disorder

Comprehensive screening investigations for the identification of patients suffering from inborn errors of metabolism are car ried out or planned, in many countries. Routine investigation of the urine of all infants for reducing substances will, as described, also act as a screening procedure for hereditary galactosaemia, the procedure mentioned above is in accordance with one of the programmes designed by the American Academy of Pediatrics [6]. Ideally routine investigation of transferase activity should be used in the screening for hereditary galactosaemia but in practice this is unfeasible It has been at tempted to use Guthrie s screening techni que for phenylketonuria [10] in the screen ing for hereditary galactosaemia [9], but as the technique is based on the pathological galactose-1 phosphate content in the erythrocytes of these patients it is possible that some cases will be missed if there is a sufficiently high galactose-1 phosphate concentration this will ensure a positive result of the test, but in such cases the patient s symptoms will have already indicated investigation for hereditary galactosaemia

We consider that the programme for the improvement of the diagnosis in hereditary galactosaemia outlined above is both practicable and capable of ensuring a reasonable degree of diagnostic certainty The principle must still be that it is necessary to bear rare diseases in mind in order to diagnose them.

## Summary

The object of this study has been to give an account of the problems associated with the diagnosis of hereditary galactosaemia. The clinical picture is described briefly and the clinical data and labora tory findings in 14 patients with galactosaemia (4 girls and 10 boys) are presented. There was no measureable galactose 1 phosphate uridyl transferase (transferase) activity in the haemolysed blood of any of the 11 surviving patients; this is specific diagnostic evidence of hereditary galactosaemia. Apart from enlargement of the liver none of the other symptoms were present in every case—even milk dyspepsia, galactosuria and proteinuria could be absent. Following early treatment with a galactose-free diet it is possible for the patients to develop normally

Two patients, who were suspected because of their family history were placed on a galactose-free diet from birth, and the diagnosis was established solely on the absence of transferase activity in the blood. In the present material the prog-

nosis was found to be good if the diagnosis was made as late as during the 4th week of life. Patients in whom the diagnosis was not established until the age of two months or more either died or showed evidence of irreversible organic damage (mental retardation, cataract involvement of the liver)

A number of findings suggest that the diagnosis is made too rarely and in many cases too late A diagnostic programme is forward, this would presumably reduce the number of missed cases and improve the prognosis in those which were discovered. The introduction of transferase measurement has meant that it is possible to hereditary galactosaemia even in the absence of clinical symptoms and signs and secondary biochemical changes and for this reason any patient in whom the disorder is suspected should, for the sake of the prognosis be placed immediately on a galactose-free diet.

The value of the measurements of transferase activity in the differential diagnosis is illustrated by the account of some cases of non-galactosaemic liver disorders occurring in the presence of galactosuria.

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CASE REPORT

# Hyperosmolarity (Hypernatremia) with Cerebral Disease

## *A Report of Two Cases in Children*

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Hyperosmolarity (hypernatremia) as a disorder associated with a variety of central nervous system diseases has received considerable attention in the recent literature None the less, it remains a diagnostic and therapeutic enigma. Only two of the cases thus far reported have been shown to have this syndrome [3 10] as defined by Welt [19]. Several other cases described y represent the same syndrome [7 15 8]. However owing to a lack of details of sodium and water balance they cannot e accepted as entirely representative In of the other cases reported there has een either significant dehydration ina lequate data to clearly support the diag is [1 4 5 6, 9 1.. 13 16, 1 22 25 ], or there has been clear evidence of nal nitrogen retention [2, 8, 23]. The cute nature of the CNS disease in many f these cases sets them apart from the well documented cases referred to bove [3 10] and makes the establishment f a definite diagnosis difficult.

The two cases of hyperosmolarity to be ported here are of interest because they were followed continuously from the onset of their illness until the time of their demise. The similarity in the course of their disease is striking and may have clinical and therapeutic implications for the management of similar acute cases of this type

## Case Reports

### *Case 1*

This 22 month old female was admitted to the intensive care unit with fever of 40°C lethargy and suspected meningococcal men ingitis. There was no evidence of shock, bleeding or focal neurologic disease on ad mission. Lumbar puncture revealed a cloudy spinal fluid containing 7000 cells/mm (78% polymorphonuclear leucocytes) and a protein of 104 mg/100 ml. Initial therapy consisted of intravenous fluids, penicillin, sulphadia zine and hloramphenicol. The latter drug was discontinued when culture confirmation of the diagnosis was obtained From the second day of hospitalization until her death her rectal temperature varied between 35 and 36°C.

Six hours after admission the patient had a generalized seizure followed by respiratory arrest Tracheotomy was done immediately she was curarized, placed on continuous assisted entilation and started on anticon vulsants. Twenty four hours after admission curare was discontinued, she was seizure free

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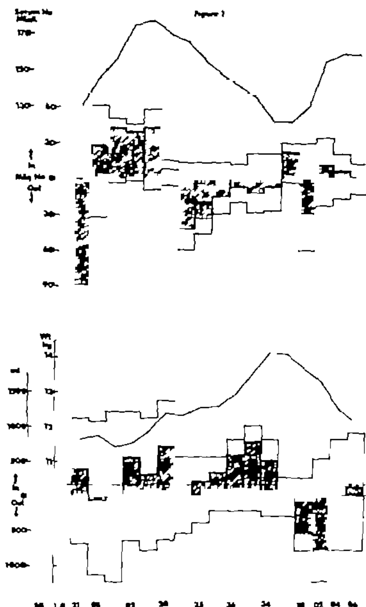


Fig 1 Patient E S The upper graph shows daily serum sodium variations, the horizontal lines indicating the range of normal values in our laboratory Sodium intake is plotted vertically upward and urinary sodium excretion downward with "net daily sodium balance" represented by the shaded area. In the lower graph water intake is plotted upward and urinary output downward with net daily water balance shaded. The broken line represents estimated insensible loss. Daily weight variations are depicted in the upper portion of this graph Daily urine specific gravity (S. G.) appears in the lower horizontal scale. Each day is represented by a single bar

flaccid and had fixed, dilated pupils. Retinal edema was present from the second t the eleventh day of hospitalization and there after the eyegrounds were deemed normal.

On assisted ventilation she maintained an average pH of 7 45, pCO of 35 mm Hg and standard bicarbonate of 25 mEq/l until her death on the nineteenth day of hospitaliza tion. She was nourished throughout her hospital course by intravenous drip and t be feedings of low protein, low electrolyte formula. Mild, generalized edema accom panied her period of rapid weight gain during the second week of hospitalization (Fig 1). Except for transient hypotension at the time of her respiratory arrest her vital signs were within normal limits until the time of her death.

Repeated BUN determinations varied between 7 and 16 mg/100 ml and total protein determinations between 4.9 and 6 4 mg/100 ml. Her blood glucose was in the normal range on repeated determinations. On the fifth hospital day during intense urinary sodium retention, her urinary aldosterone excretion was 6 μg/420 ml urine in 24 hours. This is a normal value by the method used. The detail of her fluid and electrolyt balance are summarized in Fig. 1

At autopsy the right heart was moderately dilated. The liver spleen, adrenals and kidneys were normal both grossly and microscopically There was no evidence of hemorrhage The brain was grossly edema tous, and owing to its degenerate state could not be removed in complet continuity It was soft with flat gyri and small, punctat recent hemorrhages over the brain surface There was gross venous congestion. On microscopic examination there was wide- ead necrosis, edema, diffuse fibrinous of the meninges and perivascular ocytic infiltration.

### Case 2

This previously well 9 year old female as admitted to the intensive care unit eeply comatose following a traffic accident dmission blood pressure was 55 mm Hg stolic and rose transiently to 200 mm Hg stolic Thereafter it was st ble between 90 and 120 mm Hg systolic. At the time of admission her pulse was 60 and regular By one hour after admission it was 100 and regular and remained stable at this level until her death. The pert nent clinical find ings were multiple superficial abrasions, a right temporal hematoma, a fracture f the left tibia, maximally dilated and non-reactive pupils, generalized hyperreflexia and a left Babinski sign. Four hours after admission she developed Cheyne-St kes respira tion followed by respiratory arrest. Immediate tracheotomy was done and she was maintained on assisted ventilation until her death. Urea, 1 0 g/kg was gi en at the time of admission. There was no significant change in her status until the time of her demise except for notation of mild genera lized edema following her initial period of hypernatremia (Fig 2) Rectal temperatures varied between 33 and 35°C throughout her hospital course. On assisted ventilation she maintained an average pH of 7 45 and pCO of 32 mm Hg. Aliment tion was by intra venous drip and tube feedings of a low protein, low electrolyte formula.

Repeated total protein determinations varied between 5.3 and 5.8 g/100 ml. Her BUN on the eighth hospital day was 26 mg/100 ml, on the thirteenth day 7 mg/100 ml and on the final hospital day 23 mg/100 ml Creatinine clearance was 60 ml/min/1 73 M on the eighth and seventeenth hospital days. Repeated blood glucose determinations were normal. Aldosterone excretion in 24-hour urine specimen taken between the fourth and fifth hospital days (during intense renal sodium retention) was 8.5 μg/540 ml. This is high, normal value by the method used. After five days on Aldactone 100 mg daily the 24-hour aldosterone excretion rose to 34 μg/1080 ml. This was interpreted as a normal response to Aldactone A pitressin test done on the f fteenth hospital day revealed a concentrating capacity of 670 mOsm/l while the patient was on main tenance fluid therapy Autopsy was refused by the parents. Her clinical course relative to sodium and water balance is summarized in Fig —

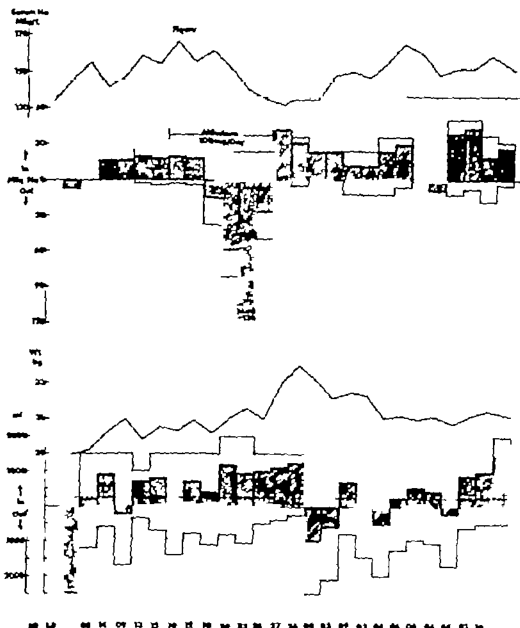


Fig. 2. Patient L. A. For explanation see Fig. 1

## Findings and Comments

It has long been recognized that a cerebral lesion might influence water and electrolyte metabolism, but it was not until the report of Allot in 1939 [1], that the association of a cerebral lesion with hypernatremia was first clearly described in man. Since that time, the role of the *CNS* in the regulation of sodium and water metabolism has been extensively studied

and some of these relationships have been recently reviewed by several authors [11 14 19 *0 4].

The available data suggest that in so far as the CNS is concerned, sodium metabolism is influenced by changes in ECF (plasma) volume. The exact location of this "volume receptor" is unknown as is its mode of action. However under certain circumstances volume expansion may lead to renal sodium diuresis and volume contraction to renal sodium retention [24]. Verney [17] in 1947 described an area of the brain responsive t changes in osmolarity Since that time this area has been shown to be involved in the regulation of water balance water retention being induced by hyperosmolarity and water loss by hypoosmolarity It has been suggested that where disorders f both volume and osmolarity coexist (i.e. hypovolemia with hyperosmolarity) volume regu latory mechanisms may take precedence [24].

Many earlier case reports of "cerebral hypernatremia probably represented sodium retention in response to inapparent dehydration and volume contraction occurring in patients with cerebral disease Some may have had impaired urinary concentrating capacity as a predisposing factor However it is also possible that some of these patients may have had an "acute cerebral hypernatremia" from which they recovered or died prior to developing a syndrome similar to that seen in chronic hypernatremia with CNS disease In cute cases, the CNS disease has usually been severe, made the diagnosis of hypernatremia difficult and precluded careful and adequate study Hyper natremia has been recognized only after several days in some

Both of the patients described in this report suffered from severe acut CNS disease and both were followed from the time of hospitalization until death. Com parison of Figs 1 and 2 reveals a similarity in the course of their illness and suggests a triphasic pattern. In one case (Case 1), this pattern was preceded by a period of urinary sodium loss in the first 1 hours of hospitalization. This was not associated with a corresponding water diuresis. In the second case the administration of urea obscured the initial changes. Both patients were, however hyponatremic on admission to the intensive care unit. It is not possible to comment on these findings.

Following this, both patients went into the first phase of their disorder This was characterised by an initial, intense urinary sodium retention followed by the devel opment of hypernatremia (and hyperosmolarity) Although this was associated with a loss of urinary concentrating capacity neither patient lost weight during this time and neither was in negative water balance. Indeed, both may have been in positive water balance. The mechanism for this sequence of events is not clear Neither patient had elevated 24-hour urinary aldosterone excretion t this time

In the second phase of their disorder both patients showed an increase in renal concentrating capacity and intense urinary sodium loss with a fall in serum sodium values to normal. Immediately thereafter there was a marked weight gain associated with a positive water balance and a fall in serum sodium values to hyponatremi levels. Throughout this second phase water balance was positive and urine output low As urinary sodium diuresis decreased water retention was ggravated, weight rose abruptly and both patients became hyponatremic and edematous.

In the third phase of their disorder inability to concentrate the urine appeared as did urinary sodium retention. It is of interest that both patients began to conserve sodium at a time when they were probably hypervolemic, as evidenced by the presence of hyponatremia weight gain, positive water balance and clinical edema. Both redeveloped hypernatremia. Although this was later associated with a negative water balance in case I and impaired concentrating capacity in both cases, both patients were hypernatremic at a time when their weights were from 1 to 1.5 kg greater than that observed on admission. Neither patient had an elevated BUN at the time hypernatremia developed Such a finding suggests that dehydration alone cannot account for the development of hypernatremia in this situation.

The management of this disorder is clinically difficult in view of the fact that hyperosmolarity develops acutely in patients where cerebral edema may be present and one desires minimal fluid administration. Moreover the nature of the primary CNS disease may obscure the presence of hypernatremia and water retention may be a significant problem. The initial clinical findings were loss of urinary concentrating capacity and intense urinary sodium retention followed by the development of hypernatremia During this first phase of the disorder minimal fluid administration as well as minimal sodium administration might be simultaneously accomplished by the intravenous administration of small amounts of glucose water alone. In the second phase of the disorder urinary concentrating capacity reappeared and a sodium diuresis occurred. This combination of events led to the development of hyponatremia with weight gain and edema. Hence with the appearance of these changes, therapy must be altered to include sodium administration. The volume of fluid administered may be judged by carefully following the patient's weight curve and urinary output. The third phase of the disorder seen in these two cases suggests that careful observation for both excessive water loss (diabetes insipidus) and sodium retention with redevelopment of hypernatremia must be maintained.

## Conclusion

The two cases reported here suffered from severe acute CNS disease and each followed a similar triphasic course Clinical balance data suggested an initial, intense renal sodium conservation as the cause of hypernatremia and did not show water loss as a contributing factor In the second phase of their illness there was positive water balance and urinary sodium diuresis. As this sodium loss in the urine ceased, water balance became increasingly positive with the development of clinical edema in both patients. In the final phase intense urinary sodium retention and hypernatremia reappeared prior to return of body weight to normal. Terminally there was suggestive evidence of diabetes insipidus in one case (Case 1)

These findings suggest a central dissociation of volume and osmolar regulation. No evidence was found for dehydration as a contributing factor to the initial hypernatremic phase in either case It is possible that those patients represent an

acute and more severe form of "cerebral hypernatremia" than that seen associated with chronic CNS disorders.


## Summary

Two cases of hyperosmolarity (hypernatremia) in children with severe CNS disease are presented. The clinical findings and sequential changes in the development of their disorder are presented and discussed briefly. It is suggested that although the acute form of this disorder may be associated with inapparent dehydration this was not the case in our patients. Intense urinary sodium retention and hypernatremia preceded the development of impaired concentrating capacity. As judged from body weight and urinary output in relation to fluid intake neither patient was hypovolemic during the initial period of hypernatremia. Some suggestions for the early diagnosis and management of this disorder are made.



## Acknowledgement

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CASE REPORT

# Chediak Higashi-Steinbrinck Syndrome

## A Survey with Report of a Case

by HÅKON BARKVE

From the Bøgeland County Hospital (Head Per Hansson), Norway

This anomaly was first reported in 1940 by Beguez Cesar and Augustin Montero in a hematological congress at the university of Havanna. Cesar published this case in 1943. In 1952 Chediak gave the first detailed hematological description. Steinbrinck (1948) and Higashi (1954) independently reported the anomaly. Some 39 cases have been published until now.

In 1959 Hansson et al. [4] reported a case from Sweden. Our patient is, as far as we know, the first recognized case in Norway.

As pointed out by the early authors the syndrome is often found in families where intermarriage is common [3]. Parents have no evidence of the disease. They are often first cousins or more distantly related [4, 1, 10]. All observations indicate that the syndrome is inherited as an autosomal gene defect.

The homozygous state, i.e., the Chediak Higashi-Steinbrinck syndrome has an equal sex distribution. The disease is usually fatal within the first five years of life but occasionally patients have reached puberty [6]. Kritzler et al. [6] identified the heterozygous carriers morphologically in a family by the presence of the granulation anomaly in the lymphocytes [6]. Sadan et al. [9] made the same observation.

### Clinical Features

All but two patients have been albinos, and one of the first symptoms recognized by the parents is photophobia. Horizontal nystagmus is often present. The skin and hair are white (oculo-cutaneous albinism). The main symptom is recurrent infection, and this is the usual cause of death [9]. A generalized lymphadenopathy with splenomegaly is common and jaundice has also been reported. Neurologic manifestations, such as convulsions, peripheral neuropathies, papilledema and mental retardation may be observed. Less common symptoms are thirst, polyuria, ascites and edema of the legs.

The recurrent infections often localized to the skin, progressively weaken the patient and the usual course is relentless. However, the course may also be more undulating.

## Hematology

The giant inclusion bodies in the leucocytes *first* brought this syndrome under discussion and constitute the only significant finding There may be one single or several bodies in one cell and they are present in all white cells of the peripheral blood as well as in their pre cursors. The granules are clearly visible in standard blood films, are red or purple usually round and surrounded by a pale halo Both size and shape may vary greatly Exceptionally the inclusion bodies may be as large as the nucleus of the cell.

*Mauri & Silingardi* [7] made a careful cytological and cytochemical study of the granules. They conclude that the abnormal granules in different cells have no com mon morphological structure. The giant granules of the myeloid cells appears qualitatively but not quantitatively close to normal. However the inclusions in the reticulumcells and lymphoid cells differ from any normal granulation of these celltypes and from known storage granules.

Sadan *et al* [9] stated that the cytoplasmic inclusions in granulocytes differ from the specific granules. Strong alkaline phosphatase activity was demonstrated in mature neutrophilic granulocytes of the peripheral blood. Acid phosphatase acti vity was found in lymphocytes, located mainly around the inclusion bodies. A recent report from White [1 ] however indicates that the giant granules may be lysosomes He suggests an abnormal permeability of the membrane surrounding these granules.

During infections the patients develop leucocytosis, often relative neutropenia and lymphocytosis. Anemia and thrombocytopenia are common in the terminal stage [11 6]. There is no explanation for the low resistance against infections It seems, however that there is no stimulating effect on the neutrophils by infectious agents [4]. The gammaglobulins are nor mal, and the patients show a normal antibody response after vaccinations.

## Postmortem studies

At autopsy the liver spleen and lymph nodes are often enlarged. Infiltration with lymphocytes and histiocytes is common in nearly all tissues. In two cases [3 8] this infiltration suggested a malignant lymphoma.

Donohue [2] and Kritzler *et al* [6] have described extensive changes in the central and peripheral nervous system. In addi tion to multiple foci with infiltrations they found evidence of degenerative processes. Kritzler *et al* also discovered the inclusions in histiocytes infiltrating the tissues as well as in renal tubular epithelium neurons of the brain, spinal cord and myenteric ganglia Johnsen *et al* [10] have recently demonstrated inclusion bodies in the limbal area, iris and choroid.

## Therapy

No treatment has so far been of any value Antibiotics have little or only temporary effect. Neither do steroids and ACTH help

## *Case Report*

The patient was a 2½ year-old girl. Before marriage, her parents came from *different* districts and consanguinity is therefore not *likely* There was no history of disease in the families. A first cousin of the patient died at the age of 4½ months, apparently from pneumonia.

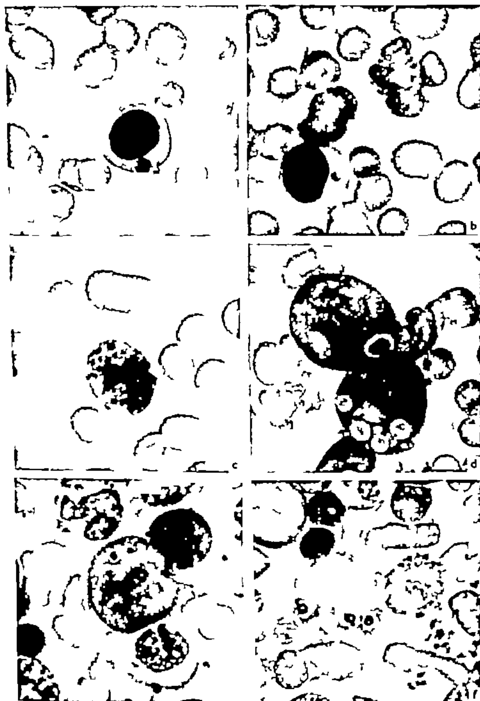



Fig 1 Different types of Chediak Higashi inclusion bodies a–c, Periph blood: (a) lymphocyte (b) neutrophile granulocyt and lymphocyt ( ) eosinoph granulocyte d–f Bone marrow (d) and ( ) myelocytes, (f) myelocyt and eosinoph granulocyt

Our patient had a brother aged 7 and a sister aged 6 years, both healthy Her father was Rh positive the mother Rh negative. No Rhesus antibodies developed during the pregnancies. The parents soon discovered the photophobia and the red pupils of the child. Otherwise the development was normal and she survived an attack of whooping cough without complications. However she was often troubled with catarrh, especially during winter time.

Three weeks before admission to hospital, she developed an infection (fissures) on the volar aspect of the fingers and these wounds did not heal. Her general condition deteriorated, and she would only take some milk and water One week prior t admission she developed a cough, high fever and enlarged lymph nodes in the neck, but after treatment with penicillin the temperature became normal.

On admission (February 1961) she was pale and seriously ill The color of the hair was extreme blond or white, the pupils red and the fundi were without visible pigment On each side of the neck there was one lymph node of cherry size and some smaller ones. These were the only enlarged lymph nodes. Coarse crepitations were heard over the lung bases. The liver and spleen were enlarged to a handbreadth below the costal margins. A chest x ray showed a soft shadow in the left apex. Hemoglobin was 76% and sedimentation rate 25 mm in one hour Red blood cells 4 13 mill. per mm Color index 0 9 White blood cells 18,700 per mm with increasing values up to 40 700 per mm *terminally Blood group A Rh positive.* Urine normal.

The bloodfilm showed anisocytosis and hypochromia. There was approximately one nucleated red cell for every two hundred white cells. Differential count. metamyelocytes 2.5%, band forms 3%, segmented neutrophils 9%, lymphocytes 85% and monocytes 0.5%.

Most of the lymphocytes were large. The nucleus was round with a loose chromatin structure. The cytoplasm was blue and often vacuolated Approximately 80% of the lymphocytes contained 1–2 large brownish-red granules, usually surrounded by a halo. The lymphocytes without inclusions were usually small. The neutrophils were also large. They contained a few blue granules and often also some large pale-red inclusion bodies.

The sternal marrow was cellular Erythropoesis was qualitatively normal but amounted only to 10% of all nucleated cells. The same inclusion bodies were found also in the whit blood cells in the marrow most strikingly in the eosinophilic myelocytes which often contained giant granules. The lymphocytes described in the peripheral blood were present also here, but they did not dominate the picture Megakaryocytes were few and seemed young

The general condition of the child deteriorated rapidly Treatment with penicillin and cortisone had no effect The fever increased and she died 10 days after the admission. Permission for autopsy was not obtained

Subsequently her parents, sister and brother were interviewed and examined N clinical evidence of disease was found *Bloodfilms* were normal and a systematic search for inclusion bodies was negative

## Summary

A short survey of the Chediak Higashi Steinbrinck syndrome with report of the first case from Norway is given. Some 30 cases have been published up to the present Characteristic features, which include *recurrent infections, hepatosplenomegaly* lymphadenopathy oculo-cutaneous albinism and a short fatal course were present in our patient. Consanguinity also frequently observed was not a feature of this case. The white blood cells *contain* giant inclusion bodies quite unlike other known granules. These may also be found outside the hemopoietic system. The disease which is lethal, is probably inherited as a recessive autosomal defect.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

# The Finnish Pediatric Society

## Meeting April 21 1965

*Andrea Prader* (Zürich): Some Experiences with Human Growth Hormone.

Pituitary growth hormone has been found to be species specific in its physico-chemical properties and physiological activity. In man only human growth hormone (HGH) and simian growth hormone have been found to be active. HGH plays a role in the anabolism of protein, mobilization of fat, oxidation of fatty acids and inhibition of glucose utilization. Its growth promoting effect stems from the anabolic action of protein. Plasma HGH concentration, which probably reflects changes in secretion rate varies with the metabolic situation. The intake of food and hyperglycemia lead to a decrease while fasting and hypoglycemia lead to an increase. In hypothalamopituitary insufficiency the normal increase following insulin induced hypoglycemia is absent.

Recently HGH has become available as an extract from human pituitaries collected at autopsy. With this material, a standardized met bolic five-day HGH test has been developed using a supposedly physiological dosage (HGH Raben mg/m daily). Using this test, the mean N-retention in 14 hypopituitary dwarfs was 114 mg/kg (SD = ±47 mg/kg) and in 15 control children 50 mg/kg (SD = ±19 mg/kg), a difference which is highly significant. Several other known HGH effects such as the increase of urinary calcium excretion and the decrease of plasma urea concentration showed no significant difference between the two groups. The test dose corrected the lack of blood glucose response following insulin induced hypoglycemia in the hypopituitary dwarfs but had little influence on the normal blood glucose response in the control children.

Sixteen dwarfs were treated with HGH Raben in a dosage of 5 mg/m twice weekly for periods of 5 months to 4 years. Three non hypopituitary dwarfs showed only very little growth acceleration. In eight hypopituitary dwarfs treated for more than one year the mean growth velocity increased from 2.7 cm per year before treatment to 8.3 cm in the first year of treatment. Four of these eight patients were treated for 3 years and more. Their mean growth velocity was 1.8 cm per year before treatment, 8.1 cm in the first year 6.6 cm in the second year and 6.2 cm in the third year of treatment. In the remaining four hypopituitary dwarfs the treatment was unsuccessful because of the development of specific HGH antibodies in high titers which blocked the metabolic and the growth promoting effects of exogenous HGH and probably also of endogenous HGH (*Lancet II* 378, 1964).

## Meeting September 18 1965

*J. Perheentupa.* Neonatal Hypoglycemia

*Märta Donner.* Infantile Spasms and Neonatal Hypoglycemia

*N. Räihä.* "Pulmonary Hypoperfusion Syndrome" (= RDS)

Meeting February 10 1966

*J P M Tizard* (London): Intraventricular Haemorrhage

Meeting March 26, 1966

with the Finnish Otolaryngologic Society

## Otitis Media in Children

*A. E. Kortekangas:* General Survey

Every inflammatory process in the Eustachian tube, tympanum, epitympanum, mastoid antrum and the mastoid air cells, is denoted middle ear inflammation. Inflammation in the tympanic cavity is usually exudative. Even at an early stage proliferative changes are typical of the air cells and often seen in the epitympanum too In young infants, however proliferative changes tend to occur in the tympanic cavity also For practical reasons the different types of acut middle ear inflammation will not be distinguished in this survey

A great number of bacteriological studies have shown that the majority of exudates in fresh cases contain pneumococci, streptococci of group A or Haemophilus influenzae. *Staphylococcus aureus is a common secon*dary invader after rupture of the tympanic membrane. The role of primary viral inflammation was discussed in the light of a few successful virus isolations from middle ear exudates. As yet, however no conclusive evidence has been produced of a primary viral inflammation in the tympanic cavity preceding the bacterial inflammation which is the predominant type in children. The main *functional failure in connection with* acut middle ear inflammation is located t the Eustachian t be Insufficient access f air t the middle ear leads either to sudden and enforced equalization of the pressure difference when nasopharyngeal secretions gain access t the middle ear or t transudation or exudation in the middle ear Among the arious symptoms of acut middle ear nflammation two are of special importance viz. decreased or lacking mobility of the drum and loss f hearing which ought to be checked in every case. The hearing loss is often so slight as to be recognizable only by a sensitive test like the whispered voice test. The age at which hearing can be reliably tested varies. When the hearing test cannot be applied, a myringotomy may sometimes be indicated for diagnostic purposes.

In the author opinion systematic anti bacterial treatment is always indicated in children. Its main effect is the prevention of complications, and this can be achieved with many available antibiotics. Considering the bacteriology of acute middle ear inflammation, preference should be given to peni cillin. It is wise to take a bacteriological culture from every patient. This is a very *useful guide for further treatment if recovery* does not occur in the expected time.

The most important procedure in the treatment of acute middle ear inflammation is early myringotomy either by puncture or the knif technique Myringotomy and suction of the secretion is the most reliable way of preventing the development f proli ferative changes in the tympanic ca ty

Every case must be followed p Complete *restoration of hearing constit tes reliable* evidence of healing In infant and young children mobility of the tympanic membrane is the most reliable sign.

Recurrent otitis may become a great problem requiring thorough examination. Even when contributing causes have been eliminated, the condition proves to be per sistent in some cases. The most important measure is to provide good drainage of the

middle ear This is usually achieved by repeated myringotomies. One important aspect of the prevention of recurrent otitis and many other diseases of the middle ear is alertness in regard t the occurrence of otitis in infants. This is a sphere in which close collaboration between paediatricians and otologists is highly desirable.

*Risto Härmä* On the Co-operation of Otologist and Paediatrician in the Treatment of Middle Ear Inflammations in Infants

Until the last decade the various branches of medicine have steadily developed towards further specialization. Meanwhile co-operation between the different specialities has not attracted sufficient attention. Otology has become an almost exclusively surgical profession, whilst in paediatrics the trend has been conservative. Such an evolution leads to the development of marginal spheres, or even vacuums, which no specialist is willing to acknowledge as his domain. This unsatisfactory state of things can be amended if the problem in question is recognized by the representatives of the specialities concerned and the borderline between them is more clearly defined.

Inflammation of the ear in infants is a typical example of an inadequately treated borderline sphere. The paediatrician is unfamiliar with otitis and the otologist with the diseases of infants. It is generally accepted, however that otitis is involved in almost 40 per cent of infections in infants. Accordingly every paediatric practitioner is confronted with otitis in hundreds of cases annually Nonetheless, almost all paediatricians lack otological training, and for this reason no uniform policy has been adopted in regard to otitis. Many paediatricians have acquired sufficient skill for diagnosing and treating otitis, but too many unfortunately remit all children with signs of otitis routinely to an otologist for consultation or treatment.

The treatment of infections in infants is completely outside the scope of the otologist, however and there is no reason why he should possess the pertinent qualifications. Since these infections as a rule are generalized, paediatric skill is required to get them under control. By contrast the otologist is competent to treat otitis in two- and three-year-old children.

In the case of uncomplicated otitis in an infant or young child, treatment by a paediatrician in a paediatric ward is much to be preferred to sending the patient to an otological outpatient department, where the risk to contract a new infection is great. But if on check-up examination of otitis it is found that healing has not occurred, or if recurrences occur co-operation with an otologist is called for

In order t improve the situation it is necessary to supplement paediatric education with otological training enabling the practitioner to take the responsibility of diagnosing treating and following up uncomplicated otitis. Since every medical student has attended a course in otology the transformation of theory into useful knowledge and routine would only require some period of training at an otological outpatient department or an otological ward for children.

This additional training ought to be supervised by an otologist so that the future paediatrician may from the outset learn the right technique and the use of the right instruments. In particular he must learn to use the forehead mirror and the pneumatic auriscope. In most cases mobility of the tympanic membrane, which can be checked with the aid of the pneumatic auriscope, is the only reliable criterion of the condition of the middle ear The most important question is not whether paracentesis should be performed or not. The main thing is that a check up examination is performed, on which it depends whether treatment should be continued or discontinued.

The prognosis for middle ear inflammation in infants in this country is dependent on the attitude adopted by the paediatricians, since this is an issue for which the otologists cannot assume the responsibility

*Heikki Kalliala* The Pediatrician s View

In all too many cases febrile illness in children is treated by a general practitioner by telephone without examination. This, of course is due to the insufficient number of physicians in our country However in fact most of the mild cases of otitis will thus be treated by antibiotic or chemotherapy only and without check of the results. Fortun tely several prominent foreign otologists seem to consider this a sufficient therapy in most mild cases.

No doubt even pediatricians perform myringotomy far too seldom This probably is due to problems of anesthesia etc and also to inexperience in otologic diagnosis. Improved practical instruction in this field would be most beneficial

## Meeting May 13–14, 1966

### Panel Discuss on "Battered Child"

Moderator J Eklund

*K Launiala*. Butterf t Load as Screening Test of Fat Absorption

*M Kekomäki:* Protein Intolerance

*Rita Asanti* Serum Alkaline Phosphatase and th Diagnosis of Rickets

The serum alkaline phosphatase activity was measured in group of infants 3 times during the first year of life The median of alkaline phosphatase values was on the average 7 B–L units showing slightly decreasing tendency with age Infants having alkaline phosphatase values of 10 B–L units or more were subjected to more detailed study but no signs of ricket or liver disease were found. Special ttention was paid t the very high values found in approximately 1.5 per cent of the samples, but no explanation for these findings was found

*Seppo Similä* Isotope Scanning of Subdural Effusion

A method of scanning subdural effusion w th $J^{131}$ hippuran was presented. By subdural puncture in cases of subdural effusion 2 ml effusion fluid is replaced wit the same amount of sterile physiologic sodium chloride which contains 1 μc $J^{131}$ hippuran. With an isotope scanning pparatus the horizontal projection of the subdural effusion is registered and on the achieved scintigram the place of sutura sagittalis and fonticul major in it normal size are marked. The subdural effusion scintigram gives good information about the place size and form of the subdural effusion.

*Antti Koivikko* Effects of Hypoxia nd Hypercapnia n the Circulati n f the N w born Lamb

The effect of hypoxia on the circulation of the newborn lamb has been studied by Cross, who found no increase in the cardiac output in response t hypoxia, contrary to the large increase in it in the adult sheep. The effects of hypercapnia on the cardiac output in the newborn lamb are not known.

In the present investigation newborn lambs were studied in hypoxia and hyper capnia using dye-dilution technique for measuring the cardiac output the circula tion time and the central blood volume In addition the ECG the rectal temperature and the respiratory rate were recorded.

In hypoxia, which was produced by 10% oxygen inhalation, the cardiac output increased even 30%, provided that there wa no metabolic acidosis. In met bolic acidosis the increase in the cardiac output was less or there was none t all. The central blood olume increased during the hypoxia period. After this period, the cardiac output and the heart rat returned quickly to thei initial levels, but the central blood volume" maintained somewhat higher level than

initially The increase in the cardiac output was produced mainly by accelerating the heart rate.

The hypercapnia was produced by 10% CO inhalation. During this period the cardiac output increased even by 40% as it is known to increase in adult humans and experimental animals (Carson) If metabolic cidosis was present, the increase was not so remarkable. Also during hypercapnia the "central blood volume was found to increase and it also remained at this level for some time afterwards, although cardiac output returned to its former level in some minutes.

*P Thuneberg* Cornelia de Lange's Syndrome

A short description was given of 4 cases of de Lange syndrome observed in Helsinki 1964–1966.

All patients were infants under 1 year 3 males and 1 female All cases presented a typical picture of de Lange's syndrome. In two cases there were severe malformations of forearm and hands (aplasia of ulna and fingers) and in all cases the hands were anomalous. Feeding difficulties, poor thriving and recurrent respiratory infections were present in all. Psychomotor retardation was evident in all cases. All had increased muscular tonus and marked opistotonus.

Chromosome analysis was normal in all. The parents were young and healthy In one case the foetal movements during pregnancy were said to be exceptionally weak. N evidence of pathological heredity was obtained except one miscarriage in third month of pregnancy All patients were born 10–14 days before term the birth weight being 1600–2670 g

One patient died at the age of 5 months in bilateral bronchopneumonia. A single umbilical artery was found at the autopsy

*K. V Parkkulainen* Results of the Treatment of Idiopathi Vesicoureteric Reflux *(to be published in 4 nales Paediatriae Fenniae)*

*Pentti Hänninen* Vectorcardiography

Three hundred vectorcardiograms of newborn infants, aged some minutes to four weeks, were examined. Frank's system of recording was used. The principal purpose was to clarify the normal findings and the dispersion of them in various newborn age groups, and separately in full term and premature infants. Attention was also paid to the influence of asphyxia and some other extracardiac factors on the electric action of the heart. Aware of the superiority of the vectorcardiographic technique in the diagnosis of cardiac hypertrophies and partial bundle branch blocks, as compared t the scalar electrocardiography one is entitled to expect considerable help from vectorcardiography in the early diagnosis of congenital heart disease.

In this investigation the average values of the 0.01, 0.02 0.03 0.04 and 0.05 sec instantaneous vectors and the half area vectors of the QRSaÊ loops were determined. Some examples of typical loops and differential diagnostic problems were also presented.

*Ilkka Välimäki* The Use of Prolonged Electrocardiocording (Holter Avionics) in Detecting Cardiac Arrhythmias in Children

The portable tape recording and rapid analyzing system described by Holter were used in detecting cardiac arrhythmias and following their response to treatment Continuous 24-hour recordings were made A modification of Wilson's chest leads V1 1 2 and V5 described before was used in infants and a bipolar lead C5-C5R in older children. Four case reports were presented (1) conversion of supraventricular tachycardia to normal sinus rhythm in a 4-week-old girl; (2) alternating second degree atrio-ventricular block in a 3 week-old mongoloid girl with congenital heart disease; (3) constant total atrio-ventricular block in a 5-year-old otherwise symptomfree boy; (4) constant bigeminy (each sinus beat followed by a ventricular one) in a 12 year-old otherwise healthy girl

Patterns differing from those seen in con-

ventional electrocardiography and especially difficulties in correct printing of the recorded signal on paper were discussed. Special attention was paid to new possibilities of detecting sudden attacks of cardiac rhythm and conduction disturbances arising in myocardial diseases, poisonings and electrolyt imbalance.

*I Väänä en* Automatization I D ta Processing in Hospitals

*Tertta Arajärvi* Th Follow-up Study of P tients in Children s Castle

*Maija-Liisa Koski* Some Psychiatric Aspects f Juvenile Diabetes

*H Åkerblom*

# Scandinavian Society of Pediatric Pathology

Meeting in Sigtuna Sweden, June 14–16 1966

*J L. Emery* (Sheffield, *guest*): Calcification f the Liver in th Newborn

Calcification of the interstitial tissue of the li er in the newborn is a rare but striking, finding and there ppears to be virtually no literature on the subject.

The calcification ppears to be of four types:

1 The commonest in which there are plaques of calcium embedded in a thickened capsule. This condition is usually due t intrauterine meconium peritonitis.

2. A large number of small masses of calcium in the portal areas scattered irregularly throughout the liver These are probably due to local vasculitis or small thrombi.

3. Large irregular masses sometimes involving one lobe only due to gross local tissue necrosis, probably following massive thrombosis.

4 Small isolated areas in portal areas probably due t escape of bile and probably a complication of the so-called inspissated bile syndrome

In addition to these cases of interstitial calcification, there are occasional instances in which there is calcification of the hepatic veins associated with hepatic venous thrombosis. These cases, in our experience, ha been of post-natal origin.

The calcification in children in this age period develops extremely rapidly and does not necessarily indicate disease of more than a week duration.

Examples of the above types of lesion are given.

*L. Lidberg* (Stockholm) Veno-occlusive Disease in the Newborn

Grass-eating animals which consume feed contaminated with plants belonging to the families Crotalaria and Senecio often develop an obliterative inflammation of the veins of the liver This disease, as has been experimentally demonstrated, is caused by well defined substances, the pyrrolizidine alkaloids. The disease called Veno-occlusive disease, has also been reported in man, mainly from African regions where these special alkaloids are included in popular remedies called bush-tea In my own experiments it was possible to induce changes in the endothelium of the liver eins in the litters of female rats which had been given the alkaloids Monocrotaline and Retrorsine By means of intravital staining with trypan blue these lesions could also be demonstrated in rat litters even though the liver of the mother was entirely normal. It could also be shown that non pregnant rats were consider

ably more sensitive to the alkaloids than were pregnant rats, and that the non pregnant females were more resistant to the alkaloids than were the male rats. These findings are consistent with the findings of other investigators who have demonstrated that the female sex hormones exert a protective effect against the pyrrolizidine alkaloids.

Pulmonary arteritis was also observed following massive pyrrolizidine poisoning. In pregnant rats it was possible to demonstrate that the severity of the pulmonary vascular changes were inversely proportional to the liver damage. In animals with widespread necrosis of the liver parenchyma no pulmonary vascular changes were observed while pulmonary vascular changes were observed in animals in which the liver was intact. It may be assumed, therefore that the toxic substances are altered within the liver parenchyma to inactive forms. When the liver parenchyma itself is destroyed this chemical alteration cannot take place.

*W. L. Donahue (Toronto, guest). Lesions in the Central Nervous System Associated with Inborn Errors of Amino Acid Metabolism*

Manifestations of brain damage have been reported in at least 18 different types of inborn errors of amino acid metabolism. Clinically the degree of involvement has varied from relatively mild effects, such as speech defects in histidinemia, to the well-known severe mental retardation of phenylketonuria.

There are relatively few descriptions of the lesions of the central nervous system in this particular family of diseases. In the reports in which there has been mention of the changes in the central nervous system, the term "spongy degeneration" or some analogous designation has been frequently used. This type of lesion has been described in Oasthouse urine disease, Maple syrup urine disease, Homocystinuria and $\beta$-Alaninemia.

In post mortem material from the Hospital for Sick Children, Toronto, Canada, in addition to those already reported "spongy degeneration" of the myelinated portion of the central nervous system has been found in other errors of amino acid metabolism. These are phenylketonuria—1 case; hyperglycinemia—3 cases; and tyrosinemia—3 cases.

This so-called "spongy degeneration" is the result of hydropic or microcystic changes in the myelin sheath. The material encompassed by the cyst-like spaces was not identified. Having regard for the age of the patient, there appeared to be some deficiency of myelin formation. A limited number of special stains did not reveal the presence of abnormal metabolic products. Varying degrees of gliosis was sometimes present. As all of the studies were retrospective no specific pattern of involvement of the central nervous system could be established. The severity of the lesion varied from case to case and from disease to disease. It appeared to be most devastating in the three cases of hyperglycinemia. These all died within one to two weeks of birth and the widespread and intense involvement of the central nervous system was probably a major factor in their demise.

In our cases of inborn error of amino acid metabolism, in addition to the spongy degeneration of the central nervous system, associated changes, not infrequently encountered, were cirrhosis of the liver—diffuse in the infant and nodular in the older children—dilatation of the proximal convoluted tubules of the kidney and hypertrophy of the islets of Langerhans. These later findings were also present in four cases of neonatal hepatitis with cirrhosis of undetermined type. Two of these were sibs. In these four cases, the changes in the central nervous system were equivocal.

Spongy degeneration of the central nervous system is seen in its most extreme form in Canavan's disease. Three such cases were available for study. In none was there any significant visceral lesion; particular attention was paid to the liver, kidney and pancreas. Two of the cases of this disease were in sibs. A third living affected infant in this

family was examined for abnormal urinary amino acids and none was found

It would seem that the damage to the central nervous system frequently encountered in inborn errors of amino acid metabolism, is the result of some abnormality of synthesis and/or homeostasis of the myelin. Because of the similarity of the pathological changes in several members of this particular family of disease there is a possibility of a common underlying mechanism.

There is also a similarity in the lesion encountered in the liver kidney and pancreas in number of these diseases.

*B G. Ockenden* (London; *guest*): Two Cases of Wolman Disease

Wolman's disease is a recently described lipidosis in which triglycerides and cholesterol are deposited in various tissues and in which the adrenals are extensively involved and calcified. It is sometimes familial, is manifest in the neonatal period or early infancy by low-grade fever diarrhoea and vomiting hepatosplenomegaly without lymphadenopathy or central nervous system signs, and terminates fatally in a few weeks or months.

The first three cases in sibs were reported from Israel (Abramov *et al.*, *Am J Dis Child* *91* 282, 1956; Wolman *et al. Pediatrics, 28* 742, 1961) and another three cases have been reported from the U.S.A. (Crocker *et al. Pediatrics 35* 627 1965).

Two further examples of this disorder have now been confirmed in England. The infants were not related and were of Irish and English extraction respectively. They were of different sex and had unaffected sibs. The clinical manifestation, the results of investigations, biopsy and autopsy findings, and chemical analyses (Prof. J M. Cumings, Institute of Neurology London) were described.

To be published in full.

*A E. Claireaux* (London; *guest*): *Krabbe* Disease

Material from 3 cases of Krabbe disease was studied. Two of the affected children were siblings. The first sibling died at the age of one year the second sibling died at the age of six months. The third child, who was not related to the other children, died at eighteen months of age

Histological examination of the brain showed demyelination of the centrum semiovale and internal capsule the major tracts in the brain stem, and demyelination of cerebellum and spinal cord. The arcuate fibres in the brain were spared. In addition to demyelination the affected areas showed severe gliosis, and numerous large and small globoid cells were present. These cells were mainly concentrated round small blood vessels. Neuronal loss was observed in the cortex and to a greater extent in the basal ganglia.

Histochemical studies were done on the available tissue The material in the globoid cells gave a strong reaction with acid phosphatase and was P.A.S. positive It gave a weakly positive reaction with Sudan Black and was faintly metachromatic with Toluidine Blue A positive reaction was also obtained with 5-nucleotidase and with Colloidal iron. These reactions appeared to indicate that the material was not a phospholipid nor a ganglioside and thus did not in any way resemble the material stored in Niemann Pick disease

Electron microscopy showed the material to have a diffuse granular appearance and to be quite unlike the coiled pattern found in Niemann-Pick disease and in Tay-Sachs disease. Numerous degenerated myelinated fibres were seen and large reactive astrocytes. The globoid cells were easily identified on account of their large size. Some contained laminated structures resembling myelin.

The disorder would seem to be a lysosomal one but the exact nature of the stored material and the precise origin of the globoid cells is still to be determined.

*D Bain* (Edinburgh, *guest*): Normal/Trisomy C Mosaicism in an Infant

A normal/C trisomy mosaic karyotype has been found in an infant aged 3 months,

Attention was drawn to the similarity between the facial appearance of this infant and that of an infant with the same karyotype, previously described by Pfeiffer Other cases in whom this karyotype has been found have varied from the apparently normal individual t the infant with mul tiple congenital malformations. Because of the difficulties in identifying individual members of the C group chromosomes it is most important that any clinical condition in which these chromosomes are implicated should be recorded and any similarities of the facial appearance noted.

*P L. Masters* (Perth, *guest*) Trisomy 18 with Visceral Haemosiderosis

*The case history of a baby girl with the* classical external and internal features of trisomy 18 was presented because heavy deposits of iron were found in the liver and well marked deposits in the thyroid, heart muscle, kidney and pars intermedia of the pituitary

Severe jaundice was present when the baby died at 6 days, but there was no liver necrosis and haemopoietic foci were small and scanty Death was due to E. coli pneu monia and meningitis.

It was suggested that the iron deposition needed the combination of a haemolytic process, possibly due to ABO incompatibility and a defect of iron transport. Reasons were given for linking this defect with the trisomic condition.

*D I Rushton (Birmingham, guest)*: Triploidy in Man

Three cases of triploidy in man were described. The first was in a 22 week infant delivered vaginally following induction for a maternal schizophrenia-like illness. The second and third cases were in bortuses of 15 and 16 weeks (by dates) The batetric histories *of the second and third cases were* not significant.

The only consistent anomalies present were bilateral colobomata. The size of the foetuses was small for their gestation and in the second and third cases they were compatible with gestation of 9 and 6, respectively

Chromosomal studies revealed caryotypes of 69 XXY 69 XXX and 69 XXX, respectively Direct preparation of amnion for nuclear sexing revealed double Barr bodies in 4 % of cells of the latter two cases but none in the former case. The nuclear size in all three cases showed a 50 % increase over normal diploid control nuclei.

The placentae of all three cases were large compared with the foetal size and in the first case the placenta showed hydatidiform change. The latter two cases showed only deficient placental vascularisation without hydropic degeneration.

*The previous cases reported in the litera* ture were reviewed and the high pre-natal mortality noted.

The mechanisms of production of triploidy were briefly discussed.

*R. Derom* (Gent, *guest*) Respiratory Dist ress Syndrome and Intra uterine Hypoxia

T assess the role of intra uterine hypoxia in perinatal mortality and morbidity cord blood has been analysed for its lactate and pyruvate content in a large number of deli veries. It is known since the fundamental work of Huckabee (*J Clin Invest*, *37* 244, 255, 264, 1958), that the ratio of lactate to pyruvate in blood increases when the oxygen supply to the tissues becomes inadequate.

From all cases of spontaneous delivery studied so far six infants died with respira tory distress syndrome. At necropsy four of them had typical pulmonary hyaline membranes while in the remaining two cases histological examination of the lungs showed atelectasis, edema and hemorrhage Age at death varied from 4 to 22 hours, birth weight from 1220 to 2800 grams.

The control series consisted of *00 normal spontaneous deliveries (Derom, *Am J Obstet & Gynec* *89* 241 1964). The lactate/pyru vate ratio had a mean value of 11 97 ±0.4 (standard deviation. 3 46) in the controls and of 11 8 ± 1.9 (range 8 1–20.0) in the

infants with respiratory distress syndrome

Thus the lactate/pyruvat ratio in cord blood is not elevated in the respiratory distress syndrome suggesting that intra uterine hypoxia does not play a major role in its etiology However more cases will have to be collected before any definite conclusion can be drawn.

*B Robertson* (Stockholm)· The Bronchial Artery in th Fetal and Neonatal Lung

Microangiographic and histological studies including serial sectioning were carried out on the lungs of 41 neonatal autopsy subjects. The birth weights ranged from 450 to 4250 g.

A few anastomoses between bronchial and pulmonary arteries were demonstrated in 16 .. of the subjects. The frequency seemed to be bout the same in the immature–premature subjects as in the fullterm infants.

Two types of anastomosis were demonstrated:

1. Side-to-side (H) anastomosis, in which the pulmonary and bronchial arteries are connected by transverse vessel in the form of a one-step ladder

2. End-to-side anastomosis, in which the bronchial artery "empties into the wider pulmonary artery

The diameter of the anastomoses varied between 25 and 100 microns.

Apart from the bronchial walls proper the bronchial arteries suppl the lymph nodes and the peribronchial nerves and they form the vasa vasorum of the major pulmonary vessels. The bronchial arteries of the fetus and newborn, however also consistently supply small areas of the alveolar tissue particularly in the circumhilar part of the lung. In such areas the capillary network of the alveolar walls seems to be continuous with that of the adjacent alveoli supplied by branches of the pulmonary artery Bronchial artery supply of the alveolar tissue can be regarded as focal retention of the early fetal vascular pattern in which the capillary plexus of the developing lung is connected with systemic as well as pulmonary arteries.

The bronch are also supplied by the so-called rami pulmobronchiales, i.e. intrapulmonary bronchial arteries originating from branches of the pulmonary artery These vessels could be distinguished from the true arterial bronchopulmonary anastomoses since they at least in the fetal and neonatal lung do not communicat on the precapil lary level with adjacent branches f the true bronchial arteries.

Focal bronchial-artery supply of alveolar tissue can occur in the vicinity of a ramus pulmobronchialis. In such areas the ramus pulmobronchialis apparently takes the place of the bronchial artery ramifying into the pulmonary parenchyma.

The normal occurrence of a focal bronchial rtery supply of the alveolar walls is I particular interest, since this pattern may be accentuated in cardiovascular malformations. For instance, direct bronchial-artery supply of the pulmonary parenchyma may be quite prominent feature, even neonatally in transposition of the great arteries. In such instances the abnormal pulmonary vascular pattern can be regarded as an intrauterine adaptation to the cardiovascular malformation.

*F Stenbäck* (Oul )· An Epidemic f Pneumocystis in an Establishment fo Children in Oulu, Finland

An epidemic on interstitial plasma cell pneumonia in an establishment in Oulu is presented. The series consists of 28 patients of which 17 were further investigated. A follow-up was also performed. The clinical data were analogous to those found in the literature except that the children were not premature. The possibility of an infectious factor could not be excluded, yet bacteriological and mycological investigations were negative Paper electrophoresis showed low gamma globulin levels both during the disease and –5 years afterwards. Immunoelectrophoretical investigations showed low IgA and IgM concentration both during the disease and during follow-up. The histological sections from lungs of one case showed the typical changes f interstitial plasma cell infiltration and al eolar foamy masses. Special attention was paid t the phagocytosis

and features compatible with septal and juxtaseptal intra-alveolar hemolysis of red cells and ppearance of discs corresponding to red cells mantles. The sequences of hemolysis seem to form a chain from the alveolar margin to the foam accumulation in the middle The possibility that some sort of temporary diapedesis of red blood cells into the alveoli and appearance of hemolytical factors from the reticuloendothelial elements of the interstitial tissue is discussed.

*A H Cameron and W H P Cant* (Birmingham, *guests*): Granuloma of Stomach

Gastrectomy was performed in a girl of 10 years of age with vague upper abdominal symptoms and haematemesis. Radiology had shown an umbilicated tumour in the pylorus.

Examination showed a granulomatous ulcer with many *eosinophils* and *giant cells*. The base of the ulcer contained many rounded bodies about 70 µ in diameter

The findings indicated that these bodies were not related to *Eustoma rotundatum* which Ashby *et al.* (*Brit Med J 1* 1141) 1964, had suggested as a common cause of eosinophilic granuloma of the alimentary tract The patient's father was a pork butcher and it was thought possible that the granuloma was due t Trichinella spiralis, though the rounded bodies did not conform to any known form of worm or larvae.

Dr John Emery pointed out that the rounded bodies were identical with the vegetable particles he had described in the lung (*Proc R.S.M* 53 952, 1960) and with those described by Sherman & Moran (*Am J Clin Path* 24 415 1954) in a peptic ulcer of the duodenum in a man aged 57 It was not thought likely that these vegetable bodies were the original cause of the granulomatous ulcer in the girl described and the aetiology of this remains in doubt.

*H G Kohler* (Leeds; *guest*): Malignant Teratoma in a Stillbirth

A large cervical tumour showing the typical features of teratoma was seen in an immature stillborn foetus. Secondary deposits were found in the liver and in the visceral pleura. These showed the gross and microscopical features of genuine metastases. Death was presumed to be due t the obstetric complications caused by the size of the tumour not by its malignancy No record of cervical teratoma with distant metastases evident at birth was found in the literature

*N J Brown* (Bristol, *guest*): Testicular Tumours in Children

Of 1500 tumours submitted to the Testicular Tumour Panel of the Pathological Society of Great Britain and Ireland during its first six years, 46 (3 %) occurred in children under 16 years.

*Orchioblastoma* (12 cases), the commonest, a mucus-secreting adenocarcinoma, occurs in young children (average * years) and has a poor but not hopeless prognosis. Its homogeneous structure suggests that it is not teratomatous in nature. Possible origins are from embryonic tubules or Mullerian tissue

*Embryonic para-testicular sarcoma* (11 cases) mainly affects older children (average 6 years). Prognosis is bad. Most were deemed rhabdomyosarcomas.

All but one of the 10 *teratomas* were well differentiated and probably benign. Histological diagnosis of malignancy is difficult as the presence of immature tissue which might *indicat malignancy in an adult, is of less* significance in a young child.

*Seminoma* (4 cases) can occur before puberty Youngest case was aged years.

*Malignant lymphoma* (3 cases) with characteristic intertubular infiltration was usually associated with lymphomatous disease elsewhere in the body

*Sertoli cell tumour* (2 cases) must be differentiated from seminoma, cells tend t be spindle-shaped with a tubular pattern

The remaining cases were, *spindle cell sarcoma fibroma pigmented retinoblastic tumour* and *dysgenetic gonadoma* (one each).

*T E Perry* (Llandough, *guest*): A te Reticulum Cell Leukaemia in Children

Four cases f acute reticulum cell leukaemia in children were described (*three*

males aged 13 months 22 months and 2½ years, and one female aged 9 years) Three were anaemic (Hb. 60 %, 43 „ and 40 %). The haemoglobin in the fourth case was 84 „. All had haemorrhagic manifestations consisting of petechiae on the fauces, cutaneous purpura or haemorrhages into the eyes and upper eyelids. Lymph gland enlargement was present in two, liver enlargement in three and the spleen was palpable in all four The initial white counts were 3 400, 50,900 21 000 and 5,600. "Blast cells ranging from 6–79 % were present in the peripheral blood in three cases. All four had thrombocytopenia (19 000–140 000/mm ) Chromosome abnormalities were not detected in the two cases where the examination was made. A remission lasting three years followed treat ment (blood transfusion, prednisone and methotrexat ) in the 2½ year old boy who survived for 3 years 11 months from the onset of symptoms. The remaining cases survived 8, 7 and 6 weeks from the onset of the disease.

The diagnosis was established in each case by the finding of large numbers of reticulum cells in the marrow These were irregular in shape, showed vacuolation of both nucleus and cytoplasm and possessed a characteristi cally coarse nuclear chromatin pattern (Israels, William Heineman, London, p. 107 1963). In two cases the cells were more mature possessing abundant cytoplasm, often producing pseudopodial processes and containing numerous vacuoles as well as fine red granules. Nuclear lobulation, however was unusual. Well marked megaloblastic changes were evident in one case

*J Lamrik* (Bergen): Blastoid Transformation f Lymphocytes from New born Infants

In mixed cultures containing lymphocytes rom tw individuals some cells undergo morphological transformation to large, blastoid cells which are able t divide This blastoid transformation is thought t be an immunological reaction, related to the genetic differences between the donors of the cells.

The reactivity of human cord lymphocytes was studied in mixed lymphocyt cultures and compared with the reactivity of adult lymphocytes. A significant higher degree of blastoid transformation was found in mixed cord lymphocyte cultures than in mixed adult lymphocyt cultures. In unmixed cord lymphocyt cultures a low degree of blastoid transformation was observed. Marked blastoid transformation was observed in cultures f lymphocytes obtained from infant following exchange transfusion. No direct evidence for blastoid change *in vivo* was found after exchange transfusion. The high blastoid reactivity of cord lymphocytes may indicat that blastoid transformation is a more primitive immunological reaction than the cellular changes accompanying antibody synthesis.

*A Serck Hanssen* (Osl ) Rhabd myosarcoma of Prostate

*R. Habib* (Paris; guest): Morphological Aspects f th Hemolytic and Uremi Syndrome in Children

One f the commonest causes of death in children with acut renal failure is the hemolytic-uremic syndrome In ten years we have observed forty cases of this disease and there are bout one hundred cases published.

*The clinical picture* is very well defined.

After two or three days of vomiting diar rhea and fever there is an brupt onset of hemolytic anemia, thrombocytopenia, pur pura and renal disease characterized by either anuria, or hematuria, proteinuria and renal insufficiency and neurological signs (convulsions).

Half of the cases are observed in infancy but they can occur later up t fourteen years of age The disease lasts f ve days up to eight weeks. In some cases, after an acut phase the disease seems to heal.

*The morphological find ngs* seem heterogeneous

1 The first cases of hemolytic uremic syndrome were described in the literature

under the name of Moschcowitz's syndrome or Thrombotic Thrombocytopenic Purpura. This disease is characterized by widespread arteriolar lesions. In children the kidney is always involved and glomerular lesions are constant. We observed two cases of this syndrome.

2. The second group of cases was reported by Gasser who described *cortical necrosis* in five infants one of whom had widespread vascular lesions in various organs like in Moschcowitz's syndrome.

We observed eight cases of this condition. The cortical necrosis is either bilateral and symmetric or patchy.

3. The third group of cases was reported in the literature under the name of *acute glomerulonephritis with hemolytic anemia*. In our opinion the glomerular lesions of this disease are very peculiar quite different of the lesion of post-streptococcal acute glomerulonephritis and almost specific for the disease. They are characterized by the association of arteriolar lesions (swelling of the endothelium with or without thrombosis) and glomerular alterations (thickening of the capillary walls by a spongy subendothelial deposit, with a few intracapillary thromboses, but without any proliferation of cells). We have called this lesion *Renal Thrombotic Microangiopathy*. We observed seventeen fatal cases of this form. None of them had extrarenal vascular lesions. In the thirteen curable forms of this disease the lesions were identical but fewer glomeruli were affected.

CONCLUSION

There are three different anatomical aspects of the Hemolytic-Uremic Syndromes: (*a*) Widespread vascular lesions of Thrombotic Thrombocytopenic Purpura, (*b*) Cortical necrosis; and ( ) Renal Thrombotic Microangiopathy.

One could question whether a lesion of the endothelium in the small arteries as well as in the capillaries of the glomerular tufts could not be the common denominator for these 3 aspects, more or less diffuse, more or less intense.

*H. Bouissou* (Toulouse *guest*) Ultramicroscopic Lesions of Nephro-anemic Syndromes

Six cases of nephro-anemic syndrome were studied.

*Recent lesions* are situated in the capillary wall and the mesangial axes.

The endothelium is detached, fragmented by a non homogeneous granulated substance of the same density as the plasma, less dense than the slightly modified basement membrane. This mostly brings about a narrowing of the lumen. The same substance also produces thickening of the mesangial matrix. We notice a reactive epithelial hyperactivity.

This standard alteration of medium intensity may show variations—modification of *the mesangial axis only*, concentric thickening of the capillary wall by heavy deposition of the substance with narrowing of the lumen occupied by agglomerations of red blood cells, necrosis of a loop or of the glomerular tuft (glomerulo-necrosis) with possibility of a tubular degeneration (Gasser's syndrome).

An arteriolar lesion, similar to the modifications of the glomerular capillaries but with endothelitis taking precedence over deposition of the amorphous material, can also be present.

These different categories of lesions are observed in the same kidney. The severity of the attack seems to be related to the type of lesion.

*Advanced lesions* are of several types—glomerular hyalinization, partial hyalinization of the glomerular tuft (tuft scar), diffuse modifications of the capillary wall and of the mesangial axes with the basement membrane irregularly thickened and hyperplasia or retraction of the mesangial axes.

The other parts of the nephron are normal.

We may consider that the basic lesion of the nephro-anemic syndrome is an endo-membranous exudative glomerulosis, a non-inflammatory phenomenon with deposition in the interior of the endomembranous space of a substance of the same density as plasma (its proteinic nature remains to be proved).

The evolution of the endomembranous exudative glomerulosis appears to have three stages—deposition, progression to one or several of the features described, and organization (the exudative material becomes membranelike).

*Krohn* (Helsinki): Morphologic Changes the Hydramniotic Placenta

(To be published in full in *Acta Path & Microbiol Scand*)

P *Larsen* (*Bergen*): Toxoplasmosis Presenting as Familial Nephrosis

In a family in which the parents were ed, the first two children were but the subsequent four died 16–46 after birth due to congenital nephrotic yndrome. In three infants the pupils were miotic, and one also had retention type undice. Three infants were autopsied, and brain tissue is available from two. One infant had a granulomatous encephalitis with giant cells and calcifications not incompatible with toxoplasmosis. The ther had more extensive necrotizing and granulomatous encephalitis with pseudocysts" diagnostic of toxoplasmosis. The mother blood had a marked positive hemagglutination reaction for toxoplasma antibodies (titre 1/160) but a negative dye test.

The kidneys in the three autopsied cases showed the glomerulopathy with associated tubular dislocation commonly described in the congenital nephrotic syndrome This lesion is not satisfactorily established as genetically determined.

Apart from the purely incidental occurrence of independant congenital nephrotic syndrome and toxoplasma encephalitis, these cases indicat the possibility that transplacental toxoplasmosis may cause both conditions. Vertical transmission of an infection can give the false appearance of heredity

*Björn Ivemark*

## ANNOUNCEMENTS

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# Fifteenth Northern (Scandinavian) Pediatric Congress Bergen June 28—July 1 1967

The XVth Northern Pediatric Congress will be held this summer in Bergen, Norway On this occasion, pediatricians from the five northern countries in Europe (Denmark, Finland, Iceland, Sweden and Norway) gather to exchange information on pediatric research, to discuss common problems, and also to meet good friends and colleagues.

The Scandinavian pediatric congresses have been held every third year since they were first started in 1919 In this half century the congresses have always been held in turn in Helsinki Copenhagen, Stockholm and Oslo With the congress in Bergen this year the pediatricians will for the first time gather outside the capitals of the Scandinavian countries.

Bergen is an old city founded about 1070 by King Olav Kyrre (Olav Haraldsson) The city was the capital of Norway until about 1300 and it was the biggest city in Norway until 125 years ago

Bergen is a university city The university is founded on the 140-years-old "Museum with some university activities But after World War II, a more complete university developed, including a medical faculty

The main subjects for the XVth Scan



Barneklinikke (The Children's Hospital), Bergen.

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Jan Winberg
Lars Wranne

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# Fifteenth Northern (Scandinavian) Pediatric Congress Bergen, June 28—July 1 1967

The XVth Northern Pediatric Congress will be held this summer in Bergen, Norway. On this occasion, pediatricians from the five northern countries in Europe (Denmark, Finland, Iceland, Sweden and Norway) gather to exchange information on pediatric research, to discuss common problems, and also to meet good friends and colleagues.

The Scandinavian pediatric congresses have been held every third year since they were first started in 1919. In this half century the congresses have always been held in turn in Helsinki, Copenhagen, Stockholm and Oslo. With the congress in Bergen this year the pediatricians will for the first time gather outside the capitals of the Scandinavian countries.

Bergen is an old city founded about 1070 by King Olav Kyrre (Olav Haraldsson). The city was the capital of Norway until about 1300 and it was the biggest city in Norway until 125 years ago.

Bergen is a university city. The university is founded on the 140-years-old "Museum" with some university activities. But after World War II, a more complete university developed, including a medical faculty.

The main subjects for the XVth Scan



Barnekhnikk (The Children's Hospital), Bergen.

dinavian Congress will deal with (1) Mal absorbtion in pediatrics, (2) Urinary tract infections and (3) Growth retardation. In addition, free papers will be read and scientific exhibitions will be presented. We will also include social events to strengthen intercollegial ties.

As President of the XVth Northern (Scandinavian) Pediatric Congress, I promise you that the Congress Committee will make all efforts for the Bergen Congress to achieve the main purpose Progress in pediatrics for the benefit of our children and close friendly contact between colleagues and people in our countries.

On behalf of the Norwegian Committee of the Scandinavian Pediatric Society I cordially welcome pediatricians from the five Scandinavian countries to the XVth Scandinavian Pediatric Congress in Bergen, 1967

ALFRED SUNDAL
President
XVth Northern Pediatric Congress

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# Cytological Diagnosis of Generalized Cytomegalic Inclusion Disease

*by* LARS CEDERQVIST and ÖRJAN JOHANSSON

Generalized cytomegalic inclusion disease in children is rare but there is evidence that its incidence is increasing [6 15 17] It is generally fatal and the diagnosis is frequently not established *ante mortem* The disease occurs in Europe, Asia and America [*]. The first 5 cases from the northern countries were described by Ahvenainen in Finland 1952 [1]. Since then 5 cases have been reported from Sweden [.., 5 12] and 1 from Norway [13]

This paper reports the first Scandinavian case of generalized cytomegalic inclusion disease diagnosed *ante mortem* by cytological examination of the urine.

## Report of Case

The patient female infant was the first hild of a 25-year-old primipara who had alw y been in good health except for acute salpingitis at 21 years of age. In the fourth month of pregnancy the mother was admitted t the department of gynaecology because of bdominal pain. Examination provided no explanation for the pain, which disappeared spontaneously and the patient as sent home. The pregnancy was other ise uncomplicated The infant was born 33 days before term. The placenta was not examined histologically Birth weight was 2200 g Crown-heel length was 44 cm and circumference of the head was 32 cm. At birth the child showed signs of severe asphyxia and received an Apgar score of 3 points. After parturition the child gradually developed petechiae ver the entire body The liver extended to, and the spleen almost to the level of the iliac crest. On the first day of life the Hb was 16.4 g/100 ml, serum bilirubin 17.3 mg/100 ml, and the direct reaction was positive. Thrombocytes numbered 44 000 per mm whit blood cells 60,200 and the red blood cells 3.8 mill per mm The SGPT was 67 units (Table 1) Skull x ray showed no intracranial calcifications. The ocular fundi appeared normal. EEG showed no bnormalities. Culture for *Listeria* gave no growth. The complement fixation reaction for cytomegalic inclusion disease was positi e (Doc Gun Carlstrom, Karolinska Sjukhuset Stockholm) (Tabl 2) The child was initially treated with steroids and antibiotics. Hepatosplenomegaly persisted. For the first 3 months of lif the child had repeated attacks of petechiae. After 2 months of age the number of thrombocytes invariably exceeded 90 000 per mm Physical and motorial dev lopment was initially slow but somewhat quicker from the 5th–6th month of lif At 9 months she was manifestly allergic t cow s milk and had several obstinat infections.

At 9 months the girl was slender and li ely She weighed 5200 g, length 6..5 cm, circumference of skull 40 cm, circumference of abdomen 43 cm. There were no bleeding manifest tions, however moderate hepatosplenomegaly persisted. At 9 months her

TABLE 1. *The table shows the development of the patient and the chief laboratory findings during the first 8 months of life.*

| Age | Partus | 2 d | 5 d. | 12 d | 3 w | 6 w | 10 w | 4 m | 8 m |
|---|---|---|---|---|---|---|---|---|---|
| Weight g | 2200 | | | 1900 | | 2200 | | 3000 | 4000 |
| Circumf. of skull, cm | 33 | | | | | 33 | | 38 | 39 |
| Hg g/100 ml | 10.4 | 17.0 | 10.8 | 12.3 | | 9.4 | 8.6 | 11.4 | 12.3 |
| RBC mill | 3.8 | 4.3 | 4.3 | 3.3 | 2.4 | | 2.3 | 4.4 | |
| Reticulocytes | 30 000 | 32,000 | 54 000 | 27,000 | 37,000 | | 26,000 | | |
| WBC | 60,300 | 27,300 | 20,900 | | 19,600 | | 4,600 | 18,900 | 19 000 |
| Thrombocytes | 44 000 | 80,000 | 42,000 | 76 000 | 100,000 | 80,000 | 92,000 | 162,000 | 340 000 |
| Serum bilirubin mg/100 ml | 17.3 | 26.1 | 20.7 | 16.0 | 4.1 | | | 0.6 | 0.6 |
| SGPT U | 67 | | 71 | | | | 58 | 31 | |
| SGOT U | | | | | | 174 | 129 | 66 | |

motorium was equal to that of a 3-month-old child. Skull x ray still showed no calcifications. The ocular fundi appeared normal.

## Method

From the third to the sixth month of life a total of 25 fresh samples of the patient's urine were collected for cytological examination performed in two different ways. Each sample was first centrifuged in 10 ml tubes, after which smears were made of the sediment on 4 slides. Secondly each sample was treated according to the millipore-technique described by Blanc & Gaetz [3]. With this method, 50 ml of fresh urine is deposited with an injection syringe into a chamber with a millipore filter disc 47 mm in diameter with a 0.80 μ pore size. The urine passes this filter and is collected in a flask with a negative pressure produced by running tap water (Fig. 1), while the cells persist in the filter. Buccal smears were obtained on 11 different occasions. Both the smear and the filter are fixed and stained by the Papanicolaou technique.

## Results

The diagnosis was considered positive as it fulfilled the criteria recommended by Naib [11], i.e. cells or well defined structures counted from the periphery

TABLE 2. *The table shows the serum antibodies of the patient and her mother during the first 6 months post partum.*

| Serology | Time after partus | Mother | Child |
|---|---|---|---|
| Toxoplasma | 2 d. | neg | |
| Listeria | 2 d | neg | |
| WR | 2 d. | neg | |
| CID* | 1 d. | 1/16 | 1/16 |
| CID | 6 w | 1/32 | 1/2. |
| CID | 3 m. | | 1/64 |
| CID | 6 m. | | 1/16 |

* Cytomegalic inclusion disease



Fig. 1 Filtrering apparatus. Urine pressed into *filter chamber where millipore-filter* on fritted glass base filters off all cells, while the urine sucked int the flask by negative pressure produced by running water



Fig 3

Figs. 2 and 3 Photomicrographs of a millipore-filtrat mounted on object glass and showing typical cytomegalic cells with large intranuclear inclusions surrounded by halo and distinct nuclear membrane (Papanicolaou stain 1000).



Fig 2

namely (1) cytoplasm, ( ) nucleus, (3) halo (4) inclusion body (Figs. and 3). Of those samples that were centrifuged, *only (8 ) were positive for cytomegalic* inclusion disease compared with 14 (56 ) of those treated according to the millipore technique

## Discussion

Generalized cytomegalic inclusion disease i caused by the salivary gland virus [10] whi h Smith succeeded in isolating from patient with this disease and cul turing in human fibroblasts [14]

The commonest sequelae are prema

turity bleeding from the umbilicus petechiae jaundice microcephaly chorioretinitis hepatosplenomegaly anaemia and periventricular calcification of the brain [16]. The children usually die from some incidental infection. The diagnosis was never made during life until 1952, when Fetterman [7] succeeded in finding characteristic cells on cytological examination of the urine from patients with cytomegalic inclusion disease Similar cells have since been found also in cerebrospinal fluid, in saliva and gastric fluid [8] In our case the buccal smears obtained on 11 occasions were negative. Inclusions of the type found in cytomegalic inclusion disease do not occur in other diseases [4 9]

The millipore-technique is superior to smear preparations in the diagnosis of cytomegalic inclusion disease This is because the cytomegalic cells in the urine are sparse in this disease and are more likely to be detected in the millipore filtrate which contains all the cells in 50 ml of the urine than in smears of specimens containing only a small proportion of the cells in 10 ml urine

## Summary

The first Scandinavian case of generalized cytomegalic inclusion disease diagnosed ante mortem by cytologic examination of the urine is described. Twenty-five samples of freshly collected urine were examined by both the smear technique and millipore-filter technique Characteristic cytomegalic cells were demonstrated in 8 % of the samples studied by the former method and in 56 % examined by the latter The millipore-filter technique thus appears to be a superior method of preparation for detection of the characteristic inclusion bodies

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# Acute Glomerulonephritis

## I Radiological changes of the lungs (Incidence types and relation to oedema hypertension and uremia)

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In 1960 Holzel & Fawcitt [6] reported a high incidence of radiographic pulmonary lesions among 65 children with acute glomerulonephritis. Prior to this report radiographic abnormalities of the chest had been described only in sporadic cases [8].

Four years later Kirkpatrick & Fleisher [8] noted a higher incidence of radiological findings in a study of 76 children with acute glomerulonephritis and suggested that chest findings, though not pathognomonic, might occasionally be of value in establishing the diagnosis of acute glomerulonephritis. The pulmonary lesion which appeared during the first week of illness were not usually associated with clinical signs from the respiratory system and very often cleared with the onset of diuresis.

In this communication we describe our experience on the incidence and type of radiographic pulmonary lesions in 100 children with acute glomerulonephritis; furthermore we report the relation of these findings to the main clinical and laboratory signs of the disease namely oedema hypertension and uremia.

### Material and Method of Study

223 cases of acute glomerulonephritis were admitted to the Department of Pediatrics of the University of Athens during the years 1961 1964. Of them, 100 cases were submitted t radiographic examination and were fully investigated. In the first period of study (1961–1963) only 41 out of 169 cases, were studied carefully. These patients had not been selected, but were taken at random, and classified as group A. In the second period (January 1963 to March 1964) all 59 in patient were f lly investigated and classified as group B.

All patients were examined and followed clinically by two doctors working separately. Blood pressure oedema and signs from the respiratory and circulatory systems were carefully noted. Body weight and urine output were recorded daily. A chest X-ray was made on admission; patients with positive findings were radiologically re-examined, till the disappearance f all pathological signs.

In addition to the clinical examination a number of laboratory investigations such as urine examination, hemoglobin, whit blood cell count sedimentation rate blood urea

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TABLE 1 *Age and sex distribution of acute glomerulo ephritis*

| Age in years | Male | Female | Total |
|---|---|---|---|
| 2–3 | 4 | — | 4 |
| 3–4 | 18 | 3 | 4 |
| 4–5 | 18 | 10 | 28 |
| 5–6 | 20 | 10 | 30 |
| 6–7 | 20 | 14 | 34 |
| –8 | 18 | 8 | 26 |
| 8–9 | 12 | 8 | 20 |
| 9–10 | 14 | 5 | 19 |
| 10–11 | 12 | 2 | 14 |
| 11 12 | 3 | | 5 |
| 12 13 | 7 | 5 | 12 |
| 13–14 | 5 | 2 | 7 |
| 2 14 | 149 | 4 | 223 |

Male–female ratio: 149/74 ( 1).

antistreptolysin titer throat culture serum proteins and electrolytes were also performed

In this study only cases with the typical clinical and laboratory findings f acute glomerulonephritis were included.

In Table 1 the age and sex incidence of the total number of patients with acut glomerulonephritis is shown. The age of the patients ranged from 2 14 years; the highest incidence of the disease was found between the ages of 4–8 years to which group more than 60 % of the patient belonged. After the ge of 10, a steady decline in the inci dence of the disease was observed.

The male–female ratio was 1 This find ing is in agreement with the prevailing opin ion that acut glomerulonephritis is more common in boys than in girls [3].

TABLE 3 *Types and ncidence of radiological f nd ngs of the chest in 56 cases of acute glomerulonephritis*

| Type of lesion | No. of cases | |
|---|---|---|
| 1 Alterations of pulmonary vasculature | 2 | 45 |
| 2. Alterations of pulmonary asculature with interlobar effu sion | 6 | 10 7 |
| 3. Alterations of pulmonary vasculature with signs of pulmon ary oedema (diffused haziness or localized areas of confluent density) | 15 | 26.8 |
| 4 Fluid in pleural cavity associa ted with pulmonary lesions oe dema or hyperemia | 8 | 14.3 |
| Total | 56 | 100 |

## Results

*Incidence and types of radiological find ngs*

Out of 100 patients who had been submitted to radiological examination of the chest, 56 had positive findings from the lungs As seen in Table * the incidence of pulmonary lesions in groups A and B was about the same

In order to classify the different types of pulmonary lesions ccording to the radiological findings w followed the distinc tion proposed by Holzel & Fawcitt and Kirkpatrick & Flei her with few minor

TABLE *Incidence f radiological lesions from the lung in 100 pat ts with acute glomerulonephritis*

| | Total Radiological lesions | | | Radio logical lesion | |
|---|---|---|---|---|---|
| Groups | Number | No. of cases | | No. of cases | |
| A | 41 | 4 | 58 5 | 17 | 41 5 |
| B | 59 | 3 | 54 | 27 | 45 8 |
| A B | 100 | 56 | 56 0 | 44 | 44 0 |




Figs. 1 and 2. Radiographs of the chest in case 1.—*Fig. 1* On admission. Congestion of pulmonary vessels, diffused haziness, confluent density of the right lower lobe, and effusion in the right pleura. *Fig. 2.* 3 days later

alterations The main types of lesions and their frequency are shown in Table 3

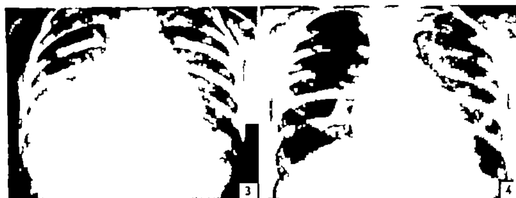
The most common type of pulmonary lesion among the 50 patients with positive lung findings was that of alterations in the pulmonary vasculature These alterations were characterized by an increase in the size of the hilar vessels, by indistinctness of the borders of the intrapulmonary vessels and by an increase in the number of visible peripheral pulmonary vessels. Alterations in the pulmonary vasculature were the only positive signs of pulmonary lesion in 27 patients (48 %) In another 6 (10 %) alterations in the pulmonary vasculature were associated with thickening of the interlobar fissure suggestive of a small collection of fluid. In 15 (27 %) radiological signs suggestive of pulmonary oedema [5] were found. In another 8 (14 %) there was a collection of fluid in the pleural cavity in addition to the pulmonary lesions.

Two types of radiological findings were interpreted as evidence of pulmonary oedema. (a) diffused haziness of both lung fields, which was considered to represent diffused pulmonary oedema (Fig 3) and (b) localized areas of confluent density suggestive of localized oedema (Fig 1).

On the initial radiograph it was difficult to differentiate confluent densities which might have been caused by consolidation from those caused by oedema. Those changes which cleared rapidly along with the disappearance of changes of the pulmonary vasculature or pleural fluid, were assumed to be caused by oedema. In these cases the rapid resolution of pulmonary consolidations a well as the absence of high fever did not favour the diagnosis of an inflammatory process of the lungs.

In most cases the lesions were unilateral affecting mainly the right lung (57 %), with only one exception affecting the left lung In a high percentage of cases (41 %) the lesions were bilateral

All patients with positive findings were followed radiologically until the complete disappearance of all pathological signs. Radiological improvement was usually observed within 3–7 days and was mainly




Figs. 3 and 4. Radiographs of the chest in case 2.—*Fig. 3.* On admission. Diffused haziness with extensive shadows of the middle and lower lobes of the right lung. *Fig. 4.* 3 days later. Considerable improvement.

related to the extent and type of lesions. In most cases it coincided with the onset of diuresis and clearance of oedema.

As a rule in most patients clinical findings from the respiratory system were lacking. Only in a few patients were clinical signs prominent these were usually the patients with fluid in the pleural cavity but even then, the extent of the radiological findings was disproportionat to the clinical signs, which were commonly very mild. In only one case (case ) with pleural effusion and pulmonary oedema was dyspnoea evident.

*Reports of illustrat ve cases*

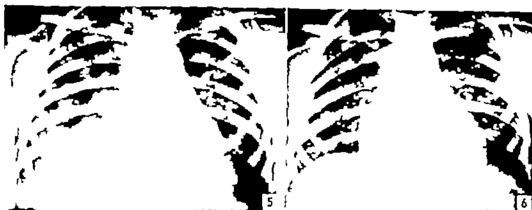
The following brief reports are examples of the main types of pulmonary lesions that have been found in patients with acut glomerulonephritis.

*Case 1*

P. A. A 9-year-old boy. 3 days before admission the patient had low grade fever with oliguria and a mild generalized oedema. Very few moist râles and decreased breathing sounds at the right base were found. Systolic arterial blood pressure was 160 mm Hg, and diastolic 110 mm Hg, blood urea 38 mg per 100 ml, red cells and casts were present in the urine. Chest x ray examination (Fig. 1) demonstrated an increase in the size of the hilar vessels, some degree of consolidation of the lower lobe of the right lung, and fluid in the right pleura. 3 days later though hypertension persisted, diuresis was est blished and the radiographic ppearances improved considerably (Fig. 2). Hypertension lasted for 10 days. Digitalis was not administered.

*Case 2*

P. G. Female 7 years old. During the last week she had anorexia, lassitude low grade fever cough and mild generalized oedema, which was most prominent on the eyelids and lower extremities. On clinical examination moist râles, and dullness at the right base were found. Dyspnoea was prominent. Systolic arterial blood pressure was 190 mm Hg and diastolic 110 mm Hg. Many red cells and casts were found in the urine blood urea was normal. Chest x rays (Fig. 3) showed extensive shadows of the right middle and lower lobes, and the presence of a small quantity of fluid in the right pleura. Diuresis was established in 3 days, while blood pressure returned t normal 5 days later. A second radiological examination on the third day from admission showed considerable improvement (Fig. 4). Digitalis was not administered.



Figs. 5 and 6. Radiographs of the chest in case 3. *Fig 5*. On admission. Diffused haziness confluent density on the right lower lobe and small collection of fluid in the right pleura. *Fig 6*. 7 days later Considerable improvement.

*Case 3*

T S Male patient 6 years old 2 days before admission the patient had hematuria and oedema of the face and lower extremities. On clinical examination nothing could be detected from the respiratory system, blood pressure was normal and blood urea 48 mg per 100 ml. Chest x rays (Fig 5) showed signs of pulmonary oedema, and small areas of consolidation of the right lung. Radiological lesions disappeared within 7 days (Fig. 6). Diuresis began on the fifth day

*Correlation of radiological findings to oedema, hypertension and uremia*

In this investigation an attempt was made to study the relation, if any of positive radiological findings of the chest to the presence of oedema hypertension and uremia.

*Oedema.*—Oedema was considered to exist in the presence of puffiness of the eyelids, swellings of the dorsum of the hands and feet and generalized swelling In most patients it was found in the form of some puffiness of the eyelids and swelling of the dorsum of the feet. The correlation of oedema to pulmonary lesions is shown in Table 4.

Of 100 patients 67 had signs of clinically detectable oedema, 47 (70 %) of them had positive findings from the lungs

The difference was more striking in the incidence of oedema between patients with negative and positive radiological findings. Oedema was present in 45.5 % of the patients of the first group compared to 84 % of the patients of the second group This difference is statistically significant ($\chi^2 = 20.2$, $p < 0.01$)

It is of interest to note here that as a rule pulmonary lesions improved considerably with the onset of diuresis, which coincided in most cases with the clearance of oedema

Radiological findings and oedema did not seem to be related to disturbances of

TABLE 4 *Correlation of oedema to radiological findings of the chest*

| Oedema | Number of cases | Radiological findings | |
|---|---|---|---|
| | | Positive | Negative |
| Without | 33 | 9 | 24 |
| With | 67 | 47 | 20 |
| Total | 100 | 56 | 44 |

TABLE 5 *Correlation of arterial blood pressure to radiological lesions*

| Arterial blood pressure, mm Hg | | Number of cases | Radiological findings | |
|---|---|---|---|---|
| Systolic | Diastolic | | Positive | Negative |
| < 100 | < 70 | 11 | 4 | 7 |
| 100–130 | 70–90 | 38 | 20 | 18 |
| < 130 | > 90 | 51 | 32 | 19 |
| Total | | 100 | 56 | 44 |

serum proteins. In this respect, we estimated the total serum proteins in 39 patients with positive and in 4 with negative radiological findings. The mean value of total proteins was 6.42 ± 0 6 g per 100 ml for the first and 6.50 ± 0 6 g per 100 ml for the second group

*Arterial blood pressure.* — Blood pressure was considered to be increased in patients where a steady decline of blood pressure was observed during the course of the disease, even when initial values were not very high. The correlation of arterial blood pressure to radiological findings is shown in Table 5.

Normal blood pressure was observed in 11 patients; 4 (30 %) of them had radiological findings. 51 patients had a definite elevation of blood pressure and pulmonary lesions were observed in 3 (64 %)

Generally there was no great difference in the incidence of hypertension between patients with positive and negative radiologic findings. Hypertension was present in 57 of the cases with positive compared to 43 of the cases with negative radiological findings. This difference is not statistically significant ($\chi^2 = 1\ 0^*$ $p$ 0.‗)

It should t least be emphasized that n patients with radiological findings suggestive of pulmonary oedema hypertension as well as generalized oedema was commonly present. It was also noted that as a rule the radiological findings improved before the arterial blood pressure was restored to normal levels.

*Urea.* The relation of blood urea levels to radiological findings is shown in Table 6 Blood urea was less than 40 mg per 100 ml in 51 patients radiologic findings were encountered in 22 (44 %), compared to 34 (68 %) out of 49 patients with urea levels above 40 mg per 100 ml

No difference in the incidence of pulmonary lesions was found in 0 patients with blood urea above 100 mg per 100 ml. The incidence of uremia was somewhat higher in patients with radiological findings. Uremia was present in 60 % of the cases with and 34 % of the cases without pulmonary lesions.

TABLE 6 *Correlation of blood urea levels t pulmonary f ndi gs*

| Blood urea mg per 100 ml | Number of cases | Radiological findings | |
|---|---|---|---|
| | | Positive | Negative |
| 20–40 | 31 | 22 | 29 |
| 40–60 | 24 | 18 | 6 |
| 60–100 | 16 | 11 | 5 |
| 100 | 9 | 5 | 4 |
| Total | 100 | 56 | 44 |

## Discussion

Radiographic abnormalities of the chest of patients with acute glomerulonephritis had been noted only in sporadic case reports prior to 1960 when Holzel & Fawcitt recorded and stressed the high frequency and the different types of radiological changes of the lungs in a series of patients

Kirkpatrick & Fleisher confirmed the above observations and reported that a much higher incidence of radiological findings could be expected. To our knowledge these are the only reports on large series of patients in the literature The results of our study on 100 patients are in agreement with those of the above authors. Thus, we were able to testify to the high incidence of radiological changes and demonstrate the different types of pulmonary lesions.

A high percentage of patients (27) developed radiographic changes of the pulmonary vasculature characterized by an increase in the size of the hilar vessels and in th number of visible peripheral pulmonary vessels as well as by indistinctness of the borders of the intrapulmonary vessels. These alterations were considered to be present mainly in those patients in whom repeated radiographic examinations of the chest disclosed a complete disappearance of the above pathological findings which had been present in the first x ray picture. Six other patients showed small interlobar effusions in addition to alteration of the pulmonary vasculature

Another group of patients had radiological findings suggestive of pulmonary oedema. These consisted of diffused haziness of both lung fields or localized areas of confluent density These lesions were encountered in the radiographic examination of 15 patients in other 8 the above findings were associated with fluid in the pleural cavities.

The pathogenesis of these lesions is still unknown. It is not yet clear whether or not the radiological findings of pulmonary oedema in acute glomerulonephritis are related to arterial hypertension, increased capillary permeability excessive renal reabsorption of sodium and water some unknown factor or a combination of the above [4 8].

Holzel & Fawcitt, considering the various facts revealed by their investigation postulated a hypothesis, which may at least offer an explanation for part of the elicited findings. Thus, the presence of the lung lesions in the first week of the illness, which coincides with the development of hydremia and hypertension, and the spontaneous resolution following the onset of diuresis suggest that they result from the accumulation of oedema. This is compatible with the clinical observation that a high percentage of the patients with pulmonary lesions had clinically d tectable oedema.

In our series, oedema was present in 83 % of the patients with radiological findings of the lungs compared to only 45 % of the patients without pulmonary lesions. This difference is statistically significant ($p < 0.01$)

In favour of this hypothesis is the fact that a spontaneous and rapid resolution of pulmonary lesions usually followed the onset of diuresis, loss of weight and clearing of oedema in most of our patients.

Furthermore in studying the disturbances of the total blood plasma and red cell volumes in patients with acute glomerulonephritis, we found, during the acute phase of the disease a considerable in-

crease of the plasma volume especially in cases with positive radiological findings, while the red cell volume remained unaltered. The plasma volume decreased considerably with the onset of diuresis and returned to normal within 3–4 weeks [7]. These findings suggest that in the initial stage of the disease a considerable degree of hydremia is present [1]. In this investigation it has also been shown that radiological findings of the lungs were not associated with disturbances in total serum proteins.

Though oedema of the lungs has been described, [2, 4] during the course of chronic renal azotemia, in the case of acute glomerulonephritis neithe uremia nor hypertension seem to be major factors in the pathogenesis of pulmonary lesions.

It is reasonable to assume that the slightly higher frequency of lung lesions in acute glomerulonephritis, observed in patients with uremia and hypertension is due to the fact that in these patients nephritis runs a much severer course than in others with minimal clinical and laboratory signs.

Though the pathogenesis of radiological findings from the lungs in cut glomerulonephritis is still obscure and not easily understood, it has already been demonstrated that these lesions are a relatively common finding of the disease. According t various authors the incidence ranges from 56–75 % of cases, and seems to be as common as uremia, hypertension and oedema.

Since radiological changes are so common in acute glomerulonephritis it would ppear reasonable that at least in some cases, a radiograph of the chest could provide useful information that may assist in the diagnosis of the disease.

## Summary

The incidence and type of radiological pulmonary lesions were investigated in 100 patients with acute glomerulonephritis. In 50 definite lung changes of varying nature and extent were found. Alterations of the pulmonary vasculature with or without interlobar effusion was the most commonly encountered radiological lesion. Radiological signs of pulmonary oedema as well as fluid in the pleural cavities were less frequently found.

Pulmonary lesions were seen during the acute phase of the disease and usually cleared up with the onset of diuresis and clearance of oedema. The majority of the children with positive findings had clinically detectable oedema.

It is however questionable if the slightly higher incidence of uremia and hypertension that have been observed in patients with positive radiological findings, is related to the pathogenesis of lung lesions.

From the above results it is at least clear that in certain cases of cute glomerulonephritis, radiological xamination of the chest may be helpful in establishing th diagnosis.

## Acknowledgement

This paper is dedicated t the memory of Professor C. Choremis, whose continuous encouragement made this study possible.

W are indebted to Dr. D. Yannakos, Associate Professor of Radiology for the interpretation of the radiological findings.

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# Thyroid Function and Plasma Tyrosine in the Neonatal Period

by K. SIERSBÆK NIELSEN and J MØLHOLM HANSEN

Recent investigations into thyroid function during the neonatal period using protein-bound iodine (PBI) butanol-extractable iodine in the serum (BEI), [131]I uptake in the thyroid gland, and tri iodothyronine uptake in erythrocytes or resins (T test) suggest that there is physiological hyperactivity of the gland during the first weeks of life [2, 3 11, 1., 16, 10 1 23]. To satisfy the requirements of ccuracy in these methods it is often necessary to use several millilitres of blood for each investigation, and there are therefore few reports of individual v riations in thyroid function during the neonatal period. Furthermore there have been no studies of thyroxine levels in the blood during this period.

It is well known that the concentration of free amino acids in the blood of full term and premature infants during the neonatal period shows a significant in crease in the concentration of the mino cid tyrosine [4, 6 8 13 15 20].

In an investigation of 17 free mino acids in the blood of d lt patient with thyrotoxicosis Melmon *et al* [14] found that only the levels of tyrosine and glut mine were raised. The relationship between thyroid function and plasma tyrosine has een confirmed by later investig tion [22].

From these findings it would seem that there might be a relationship between the physiological hyperthyroidism and the tyrosine metabolism in the neonatal pe riod. We have found it of interest to in vestigate the individual variations in thy roid function during the early neonatal period, using microdeterminations of thy roxine and dialysable thyroxine in the plasma and also to make an assessment of any possible correlation between the thyroid function and the plasma tyrosine concentration during the first days of lif

## M terial and Methods

### *Full-term i fant*

The material consisted of a total of I infants (including 2 pairs of twins) and of their mothers. All pregnancies had been uncomplicated, with the xception f a few cases f sl ght pre-eclampsia. The infant were born t term and the birth weight aried between 2500 g and 4650 g, with an average of 3350 g. Blood samples were t ken from 16 of the infants every other da during the f rst week of lif Heel blood was used and on each occasion 25–30 capillary t bes were filled, this being suff ient t prov l the minimum of 300 µl plasma necess y for the anal ses The samples were tak a he same t me of the day on all occ sions I add t on plasma from cord blood was I

TABLE 1. *Mean values (± S. D.) for plasma tyrosine, plasma thyroxine, dialysable thyroxine and free thyroxine in cord blood from 21 full-term and 16 premature infants and in blood taken within 2 hours of delivery from 7 mothers of full-term infants and 5 mothers of premature infants compared with the normal values for euthyroid adults*

The normal values are given as mean ± 2 S.D.).

| | Plasma tyrosine (µg/100 ml) | Plasma thyroxine (µg/100 ml) | Dialysable thyroxine % | Free thyroxine units |
|---|---|---|---|---|
| Normal values, adults | 11.2 ± 2.6 | 9.0 ± 4.5 | 8.0 ± 1.2 | 78 ± 44 |
| Cord blood, full-term infants | 16.0 ± 3.4 | 13.9 ± 2.7 | 7.8 ± 1.2 | 107 ± 19 |
| Cord blood, premature infants | 22.0 ± 3.9 | 12.1 ± 2.0 | 9.8 ± 1.3 | 122 ± 31 |
| Mothers of full term infants | | 19.4 ± 4.3 | 5.8 ± 0.6 | 111 ± 24 |
| Mothers of premature infants | | 20.0 ± 4.6 | 5.8 ± 1.2 | 114 ± 25 |

investigated. The first blood sample from the mothers who were investigated was taken within 2 hours of delivery and further samples were taken on the 2nd, 4th, and 6th days after delivery.

*Premature infants*

A total of 16 premature infants (including 2 pairs of twins) were investigated together with 5 of their mothers. The method used was the same as that employed in the full-term infants. The birth weights varied between 1400 and 2450 g, with an average of 1965 g. In the majority of the infants the serum bilirubin was determined on the 4th day, or at other times if indicated by the degree of icterus. There was no clinically detectable icterus in those infants in whom serum bilirubin was not determined.

*Thyroxine determinations* were carried out by the method described by Murphy [17] in a modification adjusted to the use of 100 µl plasma. Thyroxine-$^{131}$I from Abbott Laboratories and Dowex 1 × 8, 200/400 mesh resin were used. The results which are given here are corrected for the recovery of thyroxine from the plasma using 95 % ethanol. In 24 single determinations on the same sample of plasma carried out over a period of 4 weeks a value of 8.3 ± 0.9 µg/100 ml (mean ± S.D.) was obtained. The mean value for normal adults is 9.0 ± 4.5 µg/100 ml (mean ± 2 S.D.). The results of the determination of thyroxine are unaffected by any possible iodine contamination [23].

*Dialysable thyroxine* (dialysable T$_4$) was determined by the method described by Christensen [1]. 50 µl plasma was added to each chamber with 500 µl 0.5 M phosphate buffer and 5 ml physiological saline. A tracer amount of radio-active thyroxine was added to one chamber. The results are given as the percentage of the radio-active thyroxine which was added which passed through the dialysis membrane in the course of 5 hours. Dialysable T$_4$ gives an expression of the percentage of the total thyroxine which exists in free form, and it has been found to be correlated to the results of the T$_3$ tests [5]. The product of dialysable T$_4$ and the total plasma thyroxine content gives an approximate expression of the absolute amount of free thyroxine (free T$_4$), and it is this amount which is presumably crucial for the metabolism of the individual. Ten determinations were carried out on the same plasma on 4

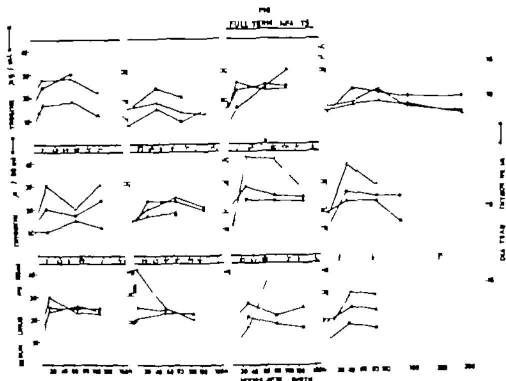


Fig. 1. Values for plasma tyrosine, plasma thyroxine, dialysable tyroxine and serum bilirubin in 12 full term infants. The values for cord blood are shown corresponding to time 0, and the remaining values according t the number of hours after birth.

different days using different batches of radio-active thyroxine and gave value of 9 4 ± 0.3 (mean ± S.D ). The mean value for dialysable T in euthyroid adults is 5.0 _ 1.3 (mean + S D ) whilst the normal mean for free T is 78 ± 48 (mean ± 2 S D ).

*Determ nations of tyrosin* were carried out by the spectrofluorometric method described by Wong *et al.* [26], which is a modification of the method described by Waalkes & Uden riend [*7]. An Aminco-Bowman spectrofluorometer was used. All analyses were car ied out in duplicate using 25 μl plasma. In 4 determinations carried out on the same lasma over period of 3 weeks value of 16 3 μg/ml (mean ± S.D.) was found. The leterminations on the plasma from the mothers and that from the cord blood were arried out as described by Waalkes & Uden riend [27]. The mean value for normal adults 11.3 3.6 μg/ml (mean S D ) [22].

## Results

### *Full-term i fants*

Table 1 contains the mean values for the plasma tyrosine T and dialysable $T_4$ and free T in cord blood from 21 full term infants. The verage values for plasma tyrosine and $T_4$ are significantly higher than the corresponding values for euthy roid adults ($p < 0.05$) The mean value for free T is also significantly raised to a value high in the normal range Fig 1 shows the individual variations in plasma tyrosine, T and dialysable $T_4$ in 12 full term infants during the first days of life There was an obvious increase in plasma tyrosine and T and dialysable T levels in all the infants, and in the majority of cases

TABLE 1 *Mean values (± S D) for plasma tyrosine, plasma thyroxine dialysable thyroxine and free thyroxine in cord blood from 21 full-term and 16 premature infants and in blood taken within 2 hours of delivery from 7 mothers of full-term infants and 5 mothers of premature infants compared with the normal values for euthyroid adults*

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| Mothers of premature infants | | 20.0 ± 4 6 | 5.8 ± 1 2 | 114 ± 25 |

investigated. The first blood sample from the mothers who were investigated was taken within hours of delivery and further samples were taken on the 2nd, 4th, and 6th days after delivery

*Premature infants*

A t tal of 16 premature infants (including pairs of twins) were investigated, together with 5 of their mothers. The method used was the same as that employed in the full term infants. The birth weights varied between 1400 and 2450 g, with an average of 1965 g. In the majority of the infants the serum bilirubin was determined on the 4th day or at other times if indicated by the degree of icterus. There was no clinically detectable icterus in those infants in whom serum bilirubin was not determined.

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TABLE 3 *Results of determinations of plasma thyroxi e dialysable thyroxine and free thyroxine in cord blood of 6 premature infants and in blood from their mothers taken within 2 hours of delivery*

| Pt. no. | Cord blood: Plasma thyroxine (µg/100 ml) | Cord blood: Dialysable thyroxine % | Cord blood: Free thyroxine units | Mothers: Plasma thyroxine (µg/100 ml) | Mothers: Dialysable thyroxine | Mothers: Free thyroxine units |
|---|---|---|---|---|---|---|
| 1 | 12.9 | 10.0 | 129 | 23.4 | 5_ | 122 |
| 2 | 15.4 | 10.7 | 163 | 19.4 | 4.1 | 80 |
| 3 | 6.9 | 13.1 | 90 | 20 0 | 6_ | 124 |
| 4 | 6.9 | 1_3 | 83 | | | |
| 5 | 7 6 | 9.9 | 75 | 13.3 | 1 | 94 |
| 6 | 10.5 | 9 4 | 99 | 24.0 | 6.3 | 131 |
| Mean ± S.D | 10.8 ± 3.6 | 10.9 ± 1.5 | 107 ± 31 | 20 0 ± 4.6 | 5.8 ± 1_ | 114 ± 3 |

peractivity of the thyroid gland during the neonatal period. We have found no significant difference between the results of the thyroid function tests in full term and premature infants expressed as units of free thyroxine However in premature infants there were lower $T_4$ values with a higher percentage of dialysable T which may perhaps be explained as the result of a reduced amount of thyroxine binding globulin. The greater tendency towards acidosis in the premature infant would also contribute to these alterations, as the binding of T to thyroxine-binding globulin is affected by pH.

The mechanism behind the increased thyroid function in the neonatal period has not been clarified. Fisher & Oddie [3] have shown that the temperature of the surroundings is of importance in the increase in PBI nd they suggest that the hyperfunction is released directly from the hypothalamic centres, and is not affected by the normal thyroxine TSH feed back system. However it is unlikely that temperature regulating mechanisms can x plain the lat increases in PBI t 0–12 weeks which have been described by Danowski [ ] and Pickering [19].

No one has previously suggested that there might be a correlation between thy roid function and tyrosine metabolism during the neonatal period. Levine *et al* [9] as early as in 1941 described an inhi bition of the metabolism of tyrosine in pre mature infants, and Kretchmer *et al* [ ] later demonstrated a reduction in the cti vity of the tyrosine transaminases in human hepatic tissue in the newborn, which was particularly marked in prema ture infants during the first weeks of lif A number of studies have revealed raised levels of plasma tyrosine during the neonatal period [6, 8 13 15, *6]. Ghadi mi &Pecora [4] have investigated th con centrations of _5 amino acids in the blood of newborn babies and found that the only significant increase wa in the tyrosine level, and these uthors seek another explanation of the lat increases in plasma tyrosine than liver enzyme immaturity Raised values of pla ma tyrosine in adults with thyrotoxicosis have been described by Soe *et al* [ 4] and confirmed in later

investigations [22]. Litwach [10] found that thyroxine inhibited the tyrosine transaminases in in vitro experiments using rat liver whilst Rivlin & Levine [20] found an increased enzyme activity in rats treated with thyroxine. The cause of the raised plasma tyrosine in patients with hyperthyroidism is as yet not elucidated but the possibility that *some part is* played by the liver enzymes cannot be excluded. On the basis of the investigations mentioned it would seem that a relationship between thyroid function and tyrosine metabolism during the neonatal period is a possibility.

Our results show that there is a correlation between the variations in plasma tyrosine and thyroid function in full term infants but no such correlation could be demonstrated in premature infants, as in the latter the greatly raised tyrosine levels which were demonstrated during the first days of life varied completely independently of the thyroxine values. In two of the premature infants who were followed for 21 and 30 days the values for both thyroxine and tyrosine were still raised at the end of this period, and according to Danowski *et al.* [2] the level of the PBI may continue to be raised for 1 weeks. It is thus possible that where raised tyrosine levels were found in premature infants after 4 weeks this might in fact be an indication of increased thyroid function. Our results do not permit any conclusions to be drawn, but suggest the possibility that the physiological hyperthyroidism during the neonatal period is a contributing factor in the increase of plasma tyrosine during the first weeks of life.

Investigation of the mothers revealed raised values for thyroxine but reduced values for dialysable thyroxine which is in accordance with the results of previous investigations of PBI and $T_3$ tests in pregnancy [11 ‒1]. However the mean free $T_4$ values were significantly increased as compared with euthyroid controls, which suggests an increase in thyroid function in late pregnancy. These findings are in agreement with those of Marks *et al.* [1*] who found a similar increase in calculated free thyroxine in mothers at term, using a different technique. The thyroxine levels in the mothers blood during delivery were significantly increased as compared with the values in cord blood, whilst the dialysable portion was reduced. A comparison of the levels of free thyroxine in cord blood and maternal blood suggests that, in the full term infants, there was free passive passage through the placenta. Where the premature infants were concerned the individual values varied to a greater extent even though the average values were almost the same. These results are in accordance with those recently published by Nayer *et al.* [18], which also suggested that the placenta is permeable to free thyroxine.

During the first days after delivery there was a fall in plasma thyroxine in the mothers, but very little increase in the dialysable part. This implies a decrease in free thyroxine and the changes mentioned above not only suggest a fall in the thyroxine binding globulin which is dependent upon oestrogens, but also a true decrease in the activity of the thyroid gland, when compared with the raised levels of free T found during delivery.

We have developed micro-thyroid function tests for use in these investigations,

and these tests are unaffected by any possible iodine contamination. As micro-analyses they have the advantage that repetition is possible without technical dif ficulty The diagnosis of hypothyroidism in childhood should be established as early as possible and we consider that repeated thyroxine micro-analyses represent the best method as yet available for diagnosing congenital myxoedema and other thyroid diseases of childhood.


## Summary

An investigation of thyroid function in the neonatal period has been made together with an attempt to assess any possible correlation between thyroid func tion and plasma tyrosine during the first days of life

Micro-analyses of plasma thyroxine and dialysable thyroxine have been used for the assessment of thyroid function, neither of these tests has previously been used in children.

A total of 1 full term infants and 7 of their mothers, and 16 premature infants with 5 of their mothers were included in the material.

Our results confirm the impression that there is physiological hyperactivity of the thyroid gland during the neonatal period. Several workers have previously demon strated a correlation between plasma tyrosine and thyroid function in adults but hitherto there has been no suggestion that there might be a similar correlation be tween thyroid function and tyrosine meta bolism in the neonatal period. In full term infants there was a correlation between the variations in plasma tyrosine and the thyroid function during the first days of life whereas the tyrosine concentrations in premature infants were very high, and apparently independent of variation in thyroid function. It is not possible to draw any definite conclusions from our results, but they suggest the possibility that the physiological hyperthyroidism of the neonatal period may be an accessory factor in the production of the hypertyrosinaemia of the first weeks of life.

The thyroid function tests which have been used are technically simple and un affected by any possible iodine contami nation. We therefore consider that these tests will be of value in the early diagnosis of congenital myxoedema nd other thy roid diseases of childhood.


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# Antibiotic Susceptibility of *Listeria Monocytogenes* and Treatment of Neonatal Listeriosis with Ampicillin

by JOHN D. NELSON,[1] SHARON SHELTON[2] and DONNA PARKS[3]

Listeriosis of the newborn presents a therapeutic problem. Most of the recommended antibiotics are unsuitable for the neonate because of toxicity or difficulties of administration. Seeliger [8] summarized the available information of *in vitro* and *in vivo* antimicrobial effectiveness and concluded that tetracyclines were the agents of choice in listeriosis. Sulfa drugs and erythromycin were also highly effective. But these are bacteriostatic agents; tetracycline carries the risk of liver damage and deposition in bone and teeth, sulfa drugs increase the hazard of kernicterus in sick, jaundiced, premature infants, and intramuscular erythromycin is irritating and cannot be given for prolonged periods. Other antibiotics are irregularly effective or have been disappointing in clinical use.

*In vitro* antimicrobial sensitivities of *Listeria monocytogenes* isolated from neonates were collected from the world literature by Ray & Wedgwood [7]. These data are sketchy and the methods of testing vary but the authors reached conclusions similar to those of Seeliger.

Nyström & Karlsson [6] tested 86 strains of animal origin by a disc diffusion method and concluded that the tetracyclines, erythromycin and streptomycin were clearly superior to other antimicrobial agents tested.

The *Listeria* strains isolated from three recent patients showed in vitro susceptibility to ampicillin and the drug resulted in clinical cures. Ampicillin is bactericidal and has proved effective in septicemic and meningitic infections of several types and, from all available information, is a safe drug in the neonate. This experience stimulated an investigation of 27 strains of *Listeria monocytogenes* isolated from humans and animals to compare *in vitro* effectiveness of ampicillin and other new penicillin-related drugs with older antibiotics and sulfadiazine.

## Materials and Methods

### *Source of cultures*

The 27 *Listeria* strains included 18 of human origin, five from animals, one from silage and three of unknown source. Geographically they represented Texas, Louisiana, Michigan, Pennsylvania, Mexico, Holland, Denmark and South America. Serotypes included type 1 (7), type 2 (2), type 3 (3), type 4a (2) and type 4b (13).

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Ten human strains were obtained through the courtesy of Dr Marion Hood, Tulane School of Medicine New Orleans, six were isolated in our laboratories in Dallas and two were obtained from Dr M. L. Gray formerly of the Veterinary Research Laboratory Montana State College Bozeman, Montana.

One animal strain isolated from a rabbit was from the American Type Culture Collection (no 4428) All other strains were obtained from Dr M. L. Gray and included two from chickens, one from mink, one from a calf one from silage and three of unknown source.

All strains were lyophilized *in vacuo* and stored at −20°C. Until lyophilization they were maintained on brain heart-infusion agar (Difco) slants at 4°C.

All strains studied conformed t the morphologic, colonial, biochemical and growth pattern characteristics of *Listeria* species. Further identification was provided by fluorescent antibody staining [5].

## Antibiotics

The following antibiotic reference standards were btained from the manufacturers: chloramphenicol (Parke Davis) ampicillin (Bristol) betacillin (Bristol) cephaloridine (Lilly) streptomycin (Pfizer) and tetracycline (Lederle). Commercial preparations of sulfadiazine (Lederle) kanamycin (Bristol) and penicillin G (Squibb) were used.

## Preparation of plates

Antibiotic concentrations were aseptically prepared by serial two-fold dilutions in oxoid sensitivity test broth (Colab) One millilite of each concentration was pipetted into 99 ml oxoid sensitivity test agar (Colab) at 48°C, t obtain final concentrations of 20, 10, 5, 2.5, 1.25, 0 64, and 0.32 μg/ml of agar In addition, lower concentrations of 0 16, 0 08, 0 04, 0 02 and 0 01 μg/ml were prepared for penicillin, ampicillin, betacillin and cephaloridine. Twenty ml of agar was poured into each plat and incubated overnight at 37°C to check for sterility All plates were sealed stored at 4°C and used within one week after preparation.

## Preparation of inocula

Cultures were tested for purity by colonial and cellular morphology on brain-heart infusion agar (Difco)

Standard plate counts were performed to determine the number of organisms per ml in 5 cc oxoid broth incubated at 37°C for 16–18 hours. Mean seed culture densities were 1.5 10⁹ bacteria per ml.

Diluted seed cultures for small inoculum testing were prepared using a platinum loop calibrated to deliver 0 001 ml. Standard plate counts were done with small inoculum seed cultures of 1:10 000 dilution prepared by adding a loopful (0 001 ml) of standard seed culture to 10 ml oxoid sensiti ity test broth and compared with plate counts from the same dilution prepared by pipetting technique. They were found to be consistently in agreement. The use of the calibrated loop provided convenience and a saving in time compared with the standard pipette-dilution method

## Plate dilution method

Both large and small inoculum testing were done using an inocula-replicator apparatus which allows simultaneous testing of 32 strains. Details of the method have been previously reported [2] The inoculum size is pproximately 0 01 ml; therefore, the actual inocula were 1.5 $10^7$ and 1.5 $10^3$ organisms with large and small inocula, respectively

Viable colony counts were made at every testing by the standard plate-count method.

Readings were made t 16–18 hours and results recorded as complet inhibition (no visible growth) partial inhibition (isolated colonies) or n inhibition (hea y growth comparable to antibiotic-free control plates)

Minimal inhibitory concentration was defined as the least amount f antibiotic concentration in μg/ml of medium that resulted in no growth.

## Results

In T ble 1 the number of *Listeria* strains with a given minimal inhibitory concen tration is shown for each antibiotic (For tetracycline and sulfadiazine those strains indicated as having minimal inhibitory concentrations of 0.32 μg/ml are actually <0.32 μg/ml since testing was not carried to lower concentrations with these drugs.)

The lowest minimal inhibitory concentrations were found with the penicillin-related drugs Tetracycline was more effective i vitro than chloramphenicol kanamycin or streptomycin.

With the smaller inocula of 1500 orga nisms the minimal inhibitory concentra tions tended to be slightly lower than those found with 15 million organism inocula for all drugs except sulfadiazine for which the difference was striking Only two strains were inhibited by sulfadiazine concentrations less than 20 μg/ml when large inocula were used but all were inhibited by 5 μg/ml (0.5 milligrams per 100 ml) or less with small inoculum testing

## Case Reports

Three recent cases of neonatal listeriosis have been successfully treated with ampi cillin as the main therapeut gent.

### Case 1

J K., 2-week-old Latin American male wa hospitalized because of irritability anorexia, and fever for one day The patient was the 3344 g product of term, uncompli cated pregnancy f a healthy 20- ear old primigravida and had done well until the present illness.

*Physical exam nation* The baby w w ll developed, irritable and h peract The body weight was 3770 g rectal empera ure 38.2°C, heart rat 160 per min t and respiratory rat 22 per minut The anterior fontanel was full but there were no other pertinent physical findings.

*Laboratory* Hemoglobin 15.3 g per 100 ml. Whit blood cell count 13,050/mm with 49% segmented neutrophils, 7 band forms, 32% lymphocytes, and 11 monocytes. The urinalysis was normal. The cerebrospinal fluid (CSF) cont ined 216 fresh red blood cell per mm and 1152 whit blood cells per mm of which 48 % were neutrophils and 52% mononuclear cells. The CSF sugar was 46 mg per 100 ml compared with simultaneous blood sugar of 82 mg per 100 ml. The CSF protein was 120 mg per 100 ml. The stained sediment revealed gram-positive rods.

*Hospital course.* Therapy was started with parenteral ampicillin (200 mg/kg/day) and kanamycin (15 mg/kg/day) The infant become f brile by the second hospital day and symptomatic improvement was rapid. *Listeria monocytogenes* was cultured from the CSF Blood cultures were sterile Kana mycin therapy was continued for a total of 10 days and ampicillin for total of 16 days. Subsequent CSF cultures were sterile and the patient wa discharged in satisfactory condition fter an 18-day hospitalization.

At follow-up examination seven months later no abnormalities were found ther than mild iron-deficiency anemia.

*Comment* This baby represents the "late-onset" type of neonatal listeriosis presumably acquired at, or shortly after birth Effective treatment was started within day after nset of symptoms and the response was excellent The *Listeria* rganisms isolated from the CSF were identified by fluorescent nt bod staining as belonging to types 1 or 3 nd by agglutination studies at the Communicable Disease Center a type 1 This strain was inhibited by 10 μg ml kanamycin and by 0 16 μg ml ampicillin with both large and small inocula

TABLE I *In vitro susceptibility of 27 strains of Listeria monocytogenes*

| Inoculum size | Antibacterial drug | > 0.01 | 0.01 | 0.02 | 0.04 | 0.08 | 0.16 | 0.32 | 0.64 | 1.25 | 2.5 | 5 | 10 | 20 | > 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Minimal inhibitory concentrations (μg/ml) | | | | | | | | | | | | | |
| 1.5 $10^{?}$ organisms | Penicillin | — | — | — | — | 3[a] | 21 | 3 | — | — | — | — | — | — | — |
| | Ampicillin | — | — | — | — | — | 10 | 17 | — | — | — | — | — | — | — |
| | Hetacillin | — | — | — | — | — | 1 | 20 | 6 | — | — | — | — | — | — |
| | Cephaloridine | — | — | — | — | — | — | 9 | 18 | — | — | — | — | — | — |
| | Tetracycline | Not tested | | | | | | 1 | 13 | 8 | 5 | — | — | — | — |
| | Chloramphenicol | | | | | | | — | — | — | 1 | 16 | 7 | 3 | — |
| | Kanamycin | | | | | | | — | — | — | 1 | 9 | 17 | — | — |
| | Streptomycin | | | | | | | — | — | — | — | — | 17 | 9 | 1 |
| | Sulfadiazine | | | | | | | — | — | — | — | 2 | — | 17 | 8 |
| 1.5 $10^{?}$ organisms | Penicillin | — | — | — | 3 | 11 | 13 | — | — | — | — | — | — | — | — |
| | Ampicillin | — | — | — | — | 2 | 15 | 10 | — | — | — | — | — | — | — |
| | Hetacillin | — | — | — | — | 2 | 8 | 17 | — | — | — | — | — | — | — |
| | Cephaloridine | — | — | — | — | — | 3 | 17 | 7 | — | — | — | — | — | — |
| | Tetracycline | Not tested | | | | | | 26 | 1 | — | — | — | — | — | — |
| | Chloramphenicol | | | | | | | — | 1 | 13 | 13 | — | — | — | — |
| | Kanamycin | | | | | | | — | 1 | 1 | 3 | 1 | 18 | — | — |
| | Streptomycin | | | | | | | — | — | — | 2 | 12 | 12 | 1 | — |
| | Sulfadiazine | | | | | | | 4 | 1 | 7 | 12 | 3 | — | — | — |

[a] Number of strains with given minimal inhibitory concentration.

*Case 2*

A. S a Negro male was the 2807 g product of term pregnancy of a healthy 40-year-old gravida 8 para 5 mother whose pregnancy was complicated by a leaking bag of waters for at least one week prior to delivery The infant was mildly depressed at birth, receiving 1 minut Apgar score of 6. There was also a maculopapular rash over the trunk and extremities which leared after two to three days. At two hours of age he was noted to be lethargic. A chest x-ray at this time revealed diffuse granulomatous type of pulmonary infiltrate; how ever there was no respiratory distress.

After blood was obtained for culture parenteral penicillin (50,000 U/kg/24 hr) and kanamycin (15 mg/kg/24 hr) therapy was initiated. At 36 hours of age the baby devel oped vomiting and abdominal distention. Unusual gray-green stool appearing to be a mixture of heavy mucus and meconium wa removed from the rectum. A lumbar puncture was done The CSF contained 286 red blood cells per mm and 18 whit blood cells per mm³ of which 11 were polymorphonuclear and 7 were mononuclear cells. The CSF sugar was 68 mg per 100 ml with a corresponding blood sugar of 86 mg per 100 ml and the CSF protein was 30 mg per 100 ml. Culture of this CSF and the original blood cultures reportedly yielded diphtheroids. Because of improvement in the infant clinical condition and the presumed failure of cultures t demonstrat "pathogens antibiotic therapy was discontinued after seven days.

The patient continued afebrile but feeding poorly until the 11th day of lif when he developed rectal temperature [illegible] 38.3°C and was again lethargic. CSF and blood were again obtained for culture prior to resumption of penicillin and kanamycin therapy The CSF culture yielded an orga [illegible]sm identified as *Listeria monocytogenes* [illegible]herapy was changed t parenteral ampicillin [illegible]n dosage of 150 mg/kg/24 hr and within [illegible] hours the infant clinical condition was much improved. Ampicillin therapy was continued for total of 23 days. Follow-up CSF cultures were steril and the patient was discharged in satisfactory condition when six weeks old

*Comment* This baby represents the "early-onset meningoepticum type of listeriosis, presumably acquired *in utero* The rash was consistent with cutaneous manifestations of this type of listeriosis The meconium obtained at 36 hours of age had been saved in a freezer because of its unusual appearance Ten days later it was examined by direct fluorescent antibody staining and numerous *Listeria monocytogenes* type 4 were identified Culture of this material revealed a heavy growth of *Listeria monocytogenes* and very light growth of *Aerobacter* sp Although the original blood and CSF cultures were discarded as diphtheroids by an inexperienced technician, they almost certainly represented *Listeria* The *Listeria* subsequently isolated from CSF was identified as type 4 by fluorescent antibody staining and confirmed as type 4b by the Communicable Disease Center agglutination studies. The organism showed the following *in vitro* sensitivities with both large and small inocula. 0 16 µg/ml penicillin, 10 µg/ml kanamycin, and 0.31 µg/ml ampicillin. The initial penicillin and kanamycin therapy had temporarily suppressed the infection but failed to sterilize the CSF

*Case 3*

D H a 3 week-old Negro male, was hospitalized because [illegible]f fever anorexia and irritability of one day duration. The infant weighed 2948 g [illegible]t birth. The mother was a 15-year-old primigra[illegible]ida who had full term, uncomplicated pregnancy The infant [illegible]ppeared normal during his nursery stay and progressed satisfactoril[illegible] [illegible]t home until the da[illegible] before admission.

*Physical examination* The weight was

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# Social Development and Declining Incidence of some Common Epidemic Diseases in Children

## *A Study of the Incidence in Different Age Groups in Stockholm*

*by* JUSTUS STRÖM

The mortality in our common infectious diseases—whooping cough, measles and scarlet fever—has rapidly declined during this century in all socially advanced countries [1–6] and as far as Sweden is concerned we hardly count on any mortality any longer in these diseases. A falling mortality may be due to many circumstances connected with improved social conditions and medical resources, resulting in better care of patients. But it may also be due to a change of the general epidemiological spectrum of the diseases, so that milder symptoms and subclinical infections occur to a higher degree

### M terial and Methods

Some years ago an analysis of this question was ttempted using the health cards of 2000 schoolchildren born in 1939 whose detailed linical records were available up t an age of 13 years. The figures of the incidence of the three abovementioned diseases seemed t be comparatively low (T ble 1) This method naturally involves errors due to faulty memory on the part of parents, wrong entries on the health cards, et On the hole however one picks up the cases w th pronounced symptoms, e. typical linical cases of the diseases.

This report gives the same analysis of 2000 schoolchildren born in 1949 and is compared with the earlier Thus ten years have passed since the previous study and one should have the possibility of forming an idea as to whether a tendency to reduced morbidity in clinically typical form really exists. W have also included a study of chickenpox in this study

If there is a reduced incidence of these diseases in children up to 13 years of age a higher incidence might be expected [illegible] this age. T get an idea of this, tw [illegible] of patients were analysed comprising [illegible] persons aged 21 30 years from 1957 [illegible] as many from 1962–63.

### Results

The figures in Table I show th[illegible] There is a sharp decline in th[illegible] especially of whooping cough [illegible] also of scarlet fever (0.2%) [illegible] (5.3%) The differences ar[illegible] significant ($p < 0.01$). The[illegible] nite difference in the fig[illegible] pox on the other hand.

*A sh ft of the ta[illegible] groups* may cause [illegible] the clinically typic[illegible] particularly to wh[illegible] to measles. Tabl[illegible]

TABLE 1 *Incidence among children born in 1939 and 1949*

| | Year of birth | Number of cases by age groups* 0–4 | 4–8 | 8–12 | Total | % |
|---|---|---|---|---|---|---|
| Whooping cough | 1939 | 841 | 403 | 38 | 1282 | 64.1 |
| | 1949 | 595 | 383 | 42 | 1020 | 51.0 |
| Measles | 1939 | 650 | 975 | 130 | 1753 | 87.8 |
| | 1949 | 593 | 684 | 173 | 1450 | 82.5 |
| Scarlet fever | 1939 | 85 | 138 | 118 | 341 | 17.1 |
| | 1949 | 71 | 83 | 64 | 218 | 10.9 |
| Chickenpox | 1939 | 445 | 609 | 196 | 1250 | 67.5 |
| | 1949 | 480 | 663 | 247 | 1390 | 69.5 |

Age groups 0–4 Infected 1939–42 and 1949–52.
4–8. Infected 1943–46 and 1953–56.
8–12 Infected 1947–51 and 1957–61

cough diminished sharply in the 0–4 age group despite which the absolute figures in the two later age groups are nearly identical. There must be a far larger number of susceptible children in these age groups in the 1949 material as so many fewer children had whooping cough in the youngest age group Calculated on the number of susceptible children in each age group the relative decline of whooping cough in the different age groups is, respectively 29.3 %, 20.2 % and 18 1 %. The greater reduction in the youngest age group is evidence of the shift in the age of contracting the disease. But this can have had no great effect on the reduced incidence of clinical whooping cough, even if one can find no mathematical expression for the fact.

The corresponding figures of the relative decline of measles in the different age groups are 9.8 %, 9.3 % and 1 6 % and scarlet fever 0 7 %, .9 % and 3 1 %. The relatively low figures in the youngest age groups indicate that a shift of the infection to higher age groups can have had no influence

The next question is whether the lower incidence of the diseases in young children led to a higher *incidence after 13 years of age*. The analysis of the two series of patients showed that the incidence in the series from 1957–68 was for whooping cough 1 4 %, measles 3.8 %, scarlet fever 3.3 %, and chickenpox 5.8 %, and in the latter from 1962–63 respectively 0.6 %, 3.4 %, 2.2 % and 4.9 %. Even if one cannot rightly calculate the total incidence of these diseases by simple addition, since the figures do not relate to the same child material, one may nevertheless say that the incidence in adulthood is comparatively insignificant and—still more important—that here again the tendency is downward anyhow not upward.

An interesting point is whether differences exist between *social classes* Social class differences have disappeared to a very large extent in Sweden. The number of children in families is small and has increasingly evened out being now highest in social class I. In this investigation the 1939 figure of children per family in class I (highest class) was 2.60 in class III

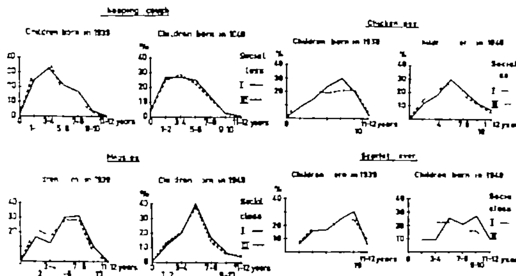


Fig. 1. Age of contracting whooping cough and measles in social classes I and III in Stockholm.

Fig. 2. Age of contracting chickenpox and scarlet fever in social classes I and III in Stockholm.

(lowest class) 2.31; the 1940 figures were 4.[illegible] and [illegible].20. In respect of housing standard the difference was greater. The number of rooms per family was 3.86 in social class I and [illegible].31 in class III in 1939; 3.84 and 1.90 in 1940.

Comparing the *age of contracting the diseases* one finds that in this respect the levelling out between the classes is nearly complete. As will be seen from Fig. 1, in 1939 there was still some difference with earlier contraction of whooping cough in class III than in class I. In the first mentioned class 552 children fell ill with pertussis and of them 386 ([illegible]0 %) contracted the disease in the 0–4 age groups. The corresponding figures in class I were 313 and 184 ([illegible]0 [illegible]). The difference (11 [illegible]) is significant ($p < 0.01$). In the 1940 material the percentages were 50.3 % and 58.8 % [illegible] the difference between the social classes had completely disappeared. The same applies to measles (Fig. 1) and chickenpox (Fig. 2); scarlet fever alone still shows a rather earlier age of contracting in class III (Fig. 2). This may possibly be due to the mode of infection. Contact is an important element in scarlet fever, which it is not with the other diseases, in which droplet and airborne infections are the dominating types.

Another interesting question arises from this result of the investigation. If there exists no difference between the social classes as regards the age of contracting the diseases, is there nevertheless any difference between the *incidence of the diseases in the classes*? To illustrate this a comparison of the incidences by social classes in the 1940 material has been done and is shown in Table 2.

The incidence of whooping cough is strikingly low in class I and the differences in relation to classes II and III are statisti-

TABLE 2 *Incidence of epidemic diseases in children born in 1949 by social classes*

| Disease | Social classes I | II | III |
|---|---|---|---|
| Whooping cough | 44.6 | 52.4 | [illegible] |
| Measles | 83.9 | 84.6 | 79.2 |
| Scarlet fever | 11.1 | 10.9 | 11.1 |
| Chickenpox | 70.8 | 70.3 | 65.4 |

cally significant ($p < 0.01$) There is no reason to presume any inadequacy in respect of diagnosis in class I, on the contrary The relatively low figures for measles and chickenpox in class III, on the other hand, are probably due to negligence of diagnosis. The almost exactly similar figures for scarlet fever undoubtedly reflect the fact that hospitalisation was compulsory for this disease

## Discussion

The practical disappearance of mortality in whooping cough, measles and scarlet fever is in keeping with the changed epidemiological spectrum for the diseases. The relatively low incidence of the clinically typical form which was found for children in Stockholm born in 1939 is further accentuated for children born 10 years later General clinical experience also provides clear support for this observation that abortive and subclinical forms have become increasingly common and the diagnosis, therefore more difficult.

What can the reasons for this be? As regards scarlet fever the widespread use of penicillin for streptococcal diseases has obviously led to a reduced incidence In the case of whooping cough there was no active prophylaxis at the time of these investigations. Passive prophylaxis with an American hyperimmune serum may have occurred to a slight extent in the 1949 material.

With the exception of scarlet fever therefore prophylactic and therapeutic measures, can have had no appreciable significance Since three infectious diseases are involved, it is difficult to find an explanation in changed virulence of the causative agents. It seems more reasonable to suppose that the persons attacked have an increasingly improved resistance. And there can hardly be any doubt that this is so The general social, economic and hygienic development, and the preventive care of mothers and children, have resulted in an extremely satisfactory state of child health.

In that way the morbidity in clinical typical diseases can be said to be a manifestation of social conditions. The lowest incidence can be expected when the age of contracting the disease is the best from the clinical point of view and the state of child health is good. The age distribution of the diseases in Stockholm seems nowadays to be rather appropriate and the social conditions are favourable for all strata of society The levelling-out of social class differences has proceeded very quickly and this is reflected in almost complete equality between the classes as regards both age of contraction and incidence.

There is, nevertheless still a difference in the whooping cough figures for 1949, the situation in social class I being markedly better than in the other two classes. This can hardly be explained by the modest chances of prophylaxis referred to above which were available to a greater

extent to class I. The result would appear to show therefore, that in regard to this disease we can go still further by further improvement of the general living standard.

I thought it of particular interest to show what a significant effect a rising social standard can have on the character of these ordinary infectious diseases. Not only does mortality diminish, but the entire spectrum of the diseases shifts towards increasingly mild, abortive forms with a corresponding reduction of the severer clinically typical cases. There is reason to dwell upon this point since proposals have been made within socially advanced countries for all kinds of universal vaccination. The demands on the vaccines, their efficiency and freedom from risk, must be all the greater the less the importance of the disease to be combated [6].


## Summary

The incidence of whooping cough, measles, scarlet fever and chickenpox has been investigated among Stockholm schoolchildren up to 13 years of age, 2000 of whom were born in 1939 and 2000 in 1949. The incidence of the first three diseases were comparatively low already in the former group (64.1 %, 87.8 %, 17.1 %) and in the latter had fallen considerably (51.0 %, 8.5 %, 10.9 %). A complementary study of the incidence among adults showed this to be low and not tending to rise. The clinically typical forms thus showed a sharply declining tendency owing to the increasingly mild nature of these diseases, which in turn is related to the growing improvement in child health and in regard to whooping cough in some degree also to a shift of the infection to a higher age. As regards scarlet fever penicillin may be presumed to have contributed to the decline. The reason for the rapid lowering of the incidence of infectious diseases is attributed to the levelling out of social classes, as a result of which both the age of contracting the diseases—except for scarlet fever—and their incidence are now the same in the three social classes. The only definite difference was that the highest social class still had a lower incidence of whooping cough. The limit of what can be achieved by general social measures without specific prophylaxis has thus not yet been reached anyhow in regard to this disease. In considering vaccination, the increasingly mild nature of the diseases in socially advanced countries should be taken into account.


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# Atypical nephronophthisis

## *A Clinico pathologic Study of Juvenile Patients without Hypotonic Polyuria*

*by* ARNE LJUNGQVIST LARS VICTORIN and JAN WINBERG

In 1951 Fanconi Hanhart Albertini, von Uhlinger Dolvio & Prader [3] de scribed a hereditary chronic nephropathy which appeared in children and adolescents and was designated familial juvenile nephronophthisis A number of later re ports published 1957–1964 have further elucidated the clinical and morphological picture of this disease [1 2, 4 5 6 8 9 11 12, 13 15]. Long lasting polydipsia hypotonic polyuria with the development of severe azotaemia in the absence of blood pressure elevation pathologic urinary findings and hydronephrosis will separate this entity from most other renal diseases. The kidneys are considerably reduced in size due to a high degree of glomerular and tubular atrophy In the medulla the tubules are tortuous and are either atrophic or hyperplastic. The basement mem branes of the loops of Henle and distal convolutions are thickened. A number of the medullary tubules are dilated and form cysts.

It is a common experience that with the recognition of a disease entity instances will soon be found where one or more of its typical features are missing It is the purpose of this paper to present two pa tients in which there was morphological and hereditary evidence of nephronophthi sis, although the typical symptoms of polydipsia and hypotonic polyuria were absent. A third patient also lacking characteristic clinical findings, but with hereditary evidence of nephronophthisis will also be discussed. It is noteworthy that the clinical features in these three patients were similar to those which appear at the age of 40–50 years in some *parents or other relatives of children with* typical juvenile nephronophthisis [10]

### Case Reports

*Case 1*

*44 04 13 K.L.* (Female, aged 17 years.)

*Heredity* Parents and one sister healthy This sister has a low creatinine learance. N other siblings.

*Early history* Congenital right facial pare sis. Deaf right ear sl ght neurogenic hearing defect for low frequences on the left side

*First signs f disease.* At the age of seven slight proteinuria was found on a routine check.

*First admission to hospital.* At the age of 8 because of persistent proteinuria

Except for primary nocturnal enuresis

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Fig 1 (Case 1) (*A*) Section from the outer medulla showing ad anced atrophy of the tubules and interstitial fibrosis. In the central and lower left aspects tortous tubules with thickened basement membranes. Bottom and left, cystically dilated tubules. AB-PAS. 80. (*B*) Section from the medulla showing tortous loops with thickened basement membranes. Bottom right, collecting duct with no evidence of basement membrane thickening. I terstitially fibrosis and moderat infiltration by mononuclear cells. AB-PAS. 200.

there was no "renal history No polydipsia nor polyuria. The bed wetting was probably of the usual central type and not connected with the renal disease

*M f nd age* Small st ture but her length and height were within normal limits. N oedema. N hypertension. No retinal hanges. Both kidney were small, of unequal size and irregular outline on i.v urograms. There was reduced glomerular function and decreased concentrating capacity with retai ned dilution bility Urine olumes 300– 00 ml/24 hours; slight proteinuria but no urinary casts or cells. Urine culture negat e Serum proteins normal Severe anaemia ppeared in the last years of lif

*Course* Attended school for nine years Participated in all common act vities but was often tired and anorectic Menarche t the age of 14. At the age f 16, more tired some what increasing thirst periodic nausea and vomiting Slightly progressive reduction of re nal parenchyma on x ra Renal osteod stro phy and calcifications of arteries in the feet

Death w th uraemia t the age of 17 Slight hypertension shortly before death, 160 110 No oedema Renal salt wasting in the last period of her lif No urinary find ings cept for slight proteinuria. Details of the linical and laboratory findings are given in T ble I and _ The increasing urine volumes and tl irst the last ear of lif may ha been d e t renal t bular calcifications (see below) as well as t the disease itself

TABLE 1. *Clinical history and results of physical examination in two patients with atypical nephronophthisis compared with one patient with typical clinical picture.*

| | | Case 1 | Case 2 | Brother of case 2 (typical case) |
|---|---|---|---|---|
| Family history positive | | No[a] | Yes | Yes |
| Sex | | ♀ | ♀ | ♂ |
| History of polydipsia and polyuria | | N | N | Yes |
| Renal history otherwise negative | | Yes | Yes | Yes |
| Renal sizes on x-ray in cm | Right | 9.5 5.5 } 8 yrs | 10 3 } 19 yrs | Unknown |
| | Left | 9.2 4.3 | 10.5 3 | |
| | Right | 5.5 2.5 } 16 yrs | | |
| | Left | 8.5 3.5 | | |
| Blood pressure | | 115/75→160/110 (17 yrs) | 140/90 | Unknown |
| Physical activity normal | | Yes | Yes | No[b] |
| Growth normal | | Yes | Yes | No[b] |
| Renal osteodystrophy | | Yes | ? | Probable |
| Eye grounds normal | | Yes | Yes | Yes |
| Auditory disturbance | | Yes | Y | N |

[a] On a family examination a sister was found to have lowered creatinine learance but was otherwise healthy.

[b] Died t the age of seventeen. Polydipsia, skeletal pains and stiffness of joints for many years before first admission to hospital. The last symptoms presumably due t renal osteodystrophy and hypocalcaemia.

*Autopsy.* There was a small intracutaneous nodule, apparently calcified, on the finger of the left hand. The lungs were moderately congested and oedematous with pleural transudation, 200 ml on each side. The heart was moderately enlarged ( 75 g) with slight left ventricular hypertrophy. No pericarditis. A mild atheromatosis was observed in the abdominal aorta and coronary arteries. All four parathyroid glands were considerably enlarged with a total weight of 3.75 g. The kidneys were very small, left 13 g and right 1 g. Cut surface pale with indistinct corticomedullary junction and occasional small cysts in the medullary area. Sections were stained for histological examinations with hematoxylineosin, van Gieson's connective tissue stain counter-stained with Weigert stain for elastic fibres and with Alcian blue-PAS.

*Microscopic exam nation.* Intracutaneous mineral salt deposition and necrosis in specimen from the fifth finger of the left hand. In all parathyroid glands considerable hyperplasia of the chief cells and water-clear cells with small strands of oxyphilic cells. Bone trabeculae in the vertebral body thin with irregular outlines and abundant intervening fibrous tissue. In the lungs foci of acute bronchopneumonia and scattered areas where alveoli were lined by structureless membranes and contained macrophages. Occasional deposits of mineral salts in the interstitial connective tissue f the lungs.

Kidneys severely altered (Fig. 1). Both cortical and medullary zones thin and papillae flattened. Most glomeruli completely scarred, others showed partial fibrosis of the capillary tuft and f brous thickening of Bowman s capsule. Some capsule spaces dilated. A few glomerular tufts apparently unaffected but showed fibrous thickening of Bowman capsule. Severe atrophy of proximal convolutions; the basement membrance moderately thickened by PAS-positive material. Loops of Henle and distal convolutions

TABLE — *Laboratory findings in two patients with atypical nephronophthisis*

| | Case 1 | | Case 2 |
|---|---|---|---|
| | Age 8 years | Age 16–17 years | Age 19–20 years |
| *Urine* | | | |
| 24 hour urine volumes, liter | < 1.0 (usually 0.5–0.7) | 1.0–2.2 | 1.5–3 (usually about 2) |
| 24 hour urine concentration spec. grav | 1 010–1.020 | — | 1 006–1.013 |
| Max. conc. capacity spec. grav | 1.021 | 1.013 | 1 013 |
| Max dilution capacity spec. grav | 1.003 | 1.008 | Not done |
| Protein ‰ | < 0.5 | < 1 | 0–0.3 |
| Erythrocytes, white cells and casts | Normal findings | Normal findings[a] | Normal findings |
| Glucose | N | — | Yes[b] |
| Aminoacids | — | — | Normal |
| Clearance ml/min | — | $C_{CR}$ 7 | $C_{IN}$ 8, $C_{PAH}$ 22 |
| *Serum* | | | |
| NPN, BUN or Creatinine mg/100 ml | NPN 35→65 | NPN 90–203 | Creat. 10–24 |
| Cholesterol mg/100 ml | 300 | 234, 271 | Not done |
| Uric acid mg/100 ml | Not done | Not done | Not done |
| Na mEq/l | 140 | 143→109 | 140 |
| Cl mEq/l | 104 | 110→80 | 85 |
| K mEq/l | 5.3 | 5.2–6.0 | 4.0 |
| Ca mg/100 ml | 11.5 | 10.8–10.4 | 5.8 |
| Phosphate mg/100 ml | 5.1 | 11.6→ 1 | 8 |

[a] Shortly before death microscopic haematuria and pyuria.
[b] 1 7–13.5 g/24 hours; blood sugar below 144.

considerably tortuous and atrophic with prominent thickening of the basement membranes. Collecting ducts either trophic or normal in appearance with no thickening of the basement membranes. In the medullary portion some small cysts lined by a single layer of cuboidal epithelium. Heavy interstitial fibrosis with small foci of lymphocytes. Intracellular mineral salt in the loops of Henle and distal convolutions. Occasional intraglomerular mineral salt deposits. Mild hyaline thickening of intrarenal arteriole walls.

*Case 2*

*H 89 20 R I* (Female aged 19 years.)

*Heredity* Parents healthy One brother died at the age of 17 with renal insufficiency N other siblings.

*First signs of disease* At the age of 19 progressively more tired. Pains in pelvic region. Haemoglobin level 6 g per 100 ml. Iron therapy without effect.

*First admission to hospital.* At the age of 19 because of iron refractory anaemia. There were no earlier symptoms of renal disease; no polydipsia, no polyuria. No hearing defect but extreme myopia (2.5 and 8.5 of glasses). Normal growth; menarche at the age of 16.

*M f ed age* Length 174 cm, weight 53 kg. N oedema. N hypertension. N retinal changes. Kidneys roentgenologically somewhat small, of about equal size and ill-defined. Reduced glomerular function, reduced concentrating capacity moderat dilution abilit Urine volumes 1.5–3 l/24 h. Slight proteinuria (mostly albumin) no urinary deposits. Urine culture negative




Fig. 2. (Case 2) (*A*) Section from the cortico-medullary area showing interstitial fibrosis and tortuosity of tubules. The distal segments of the proximal tubules are dilated without any appreciable thickening of the basement membranes (*top right*), whereas the basement membranes of the loops re clearly thickened (*arrows*). AB-PAS 80. (*B*) Section from the inner medulla showing cystic spaces lined by a single layer of epithelium. Thickened basement membranes of trophied tubules (*arrow*). AB-PAS. 80.

Electrophoretic pattern of serum proteins showed a marked increase in α globulins but was otherwise normal Hypocalcaemia was present.

*Co rse.* She was an invalid from the beginning of her symptoms until her death one year and a half later Repeated transfusions because f severe anaemia. Never any hypertension or oedema Moderat renal salt wasting during the last period of lif Still only slight proteinuria and no urinary casts nor haematuria nor leucocyturia. Death occurred during haemodialysis with evidence of a pericardial effusion. Details of the lini cal and laboratory findings are given in Tables 1 and 2.

*Autopsy* There was moderat pulmonary congestion and oedema. Blood stained effusions in the pleural (right 200 ml, left 400 ml), pericardial (200 ml) nd peritoneal cavities (1400 ml). The heart was of normal size and configuration weighing 335 g There was a minimal ortic theromatosis. Cerebral oedema was demonstrated. Both kidneys were small, equal in size and weighing 55 g each. The cut surface was pale and the cort nar row There were multiple cyst up t pea size in the med lla. Staining methods for histological examinations as f r Case 1

*Microscopic xamination.* Relevant findings in the kidneys are demonstrated in Fig. 2. Most glomerula tuft either partially

or completely scarred. Some tufts pparently intact but with fibrous thickening of Bowman's capsule A number of cystic glomeruli beneath the renal capsule. Diffuse atrophy of proximal convol tions with no evidence of basement membrane thickening. Small areas of dilated proximal convol tions. Loops of Henle and distal convolutions tortuous, either atropic or hyperplastic and with marked PAS-positive thickening of the basement membranes. Collecting duct with out appreciable alterations. Numerous cysts of arious sizes in the medulla, all lined by a single layer of cuboidal epithelium. Inter stitial connective tissue sclet tic in the cortex, but more loose in the medulla. Strands of lymphocytes, plasma cells and occasional polymorphonuclear cells in the medulla. No evidence of mineral salt deposi tion. Intrarenal vessels normal.

This patient had a brother A.G.I born in 1934. H died with uraemia in 1951 half a year aft his first admission t hospital.H had a slight proteinuria, but no urinary casts nor haematuria or leucocyturia. At an inter view with his mother 15 years after his death she described the copious thirst of her son. Every night she had had t place big jug with water beneath his bed. H was of small stature and had had skeletal pains and joint stiffness for several years before his death No autopsy was performed on this brother but his history as well as the labora tory data collected from his record are well compatible with the diagnosis of a classical nephronophthisis.

## Discussion

The two patients reported here did not show the most typical symptom of nephronophthisis which is a hypotonic polyuria that appears early in th disease and per sists until death in uraemia. Yet it seems justifiable to classify the disease in both of them as nephronophthisis. This opinion is founded on consideration of three fa tors namely postmortem examination, clinical features other than polyuria, and—thirdly—heredity Thus the structural renal lesions were identical with those we have seen in cases with the typical clinical picture [9]; the functional pattern with severe uraemia combined with retained bility to dilute urine and no or sparse urinary findings is similar to that in typical cases, the brother of Case 2 died from a renal disease with the clinical and functional characteristics of typical nephronophthisis.

In this connection it seems justifiable also to mention shortly a girl of leven years whose dominating symptom is a severe arterial hypertension. She has small kidneys, moderately decreased glomerular filtration rate slight proteinuria but no urinary deposits. In spite of extensive investigations it has not been possible to classify her disease. Attempts of renal biopsy have, however been unsuccessful so far Her mother died 40 years old in a renal disease with all the typical histological nd clinical characteristics of nephronophthisis. One may consider the possibility that this young girl represents another form of nephronophthisis with hypertension as the leading feature.

In Table 3 we have listed the most prominent clinical features in typical juvenile nephronophthisis and compared them to the findings in the two patients presented in this paper Since there is much evidence in favour of a diagnosis of nephronophthisis in these cases, although both polyuria and polydipsia are lacking we have designated their disease as typical" nephronophthisis A simila clinical picture has been encount red in some parents or other adult relatives to children with nephronophthisis [10]

TABLE 3 *Comparison of some main clinical features in typical and atypical nephronophthisis*

| | Typical | Atypical (2 cases) |
|---|---|---|
| History of polydipsia | Yes, usually of many years duration | None |
| Otherwise negative renal history | Yes | Yes |
| Hypotonic polyuria | Yes. Not sensitive to pitressin | Not remarkable, but specific gravity often below 1 010 |
| Azotaemia | Yes | Yes, but only intermittent in one |
| Proteinuria | Slight or absent | Slight–moderate |
| Urinary deposits | Absent | Absent |
| Arterial hypertension | Unusual; only in the final stage | Late sign in one patient |

Experience has shown that although a clinical diagnosis of *nephronophthisis* should present little difficulty in typical cases such patients are often diagnosed as chronic nephritis" It can be anticipated that an inadequate diagnosis is still more likely to occur in patients lacking the water losing syndrome A thorough consideration of the clinical features should, however make it possible to separate nephronophthisis from most other renal diseases. The most difficult differential diagnosis would seem to be from chronic pyelonephritis with and without renal dysplasia. In such cases the renal parenchyma may be *markedly reduced* and *the urine often* sterile as in nephronophthisis.

Morphological investigation plays an important role in the diagnosis of typical nephronophthisis. The fact that the renal lesions in our two patients, did not deviate from those seen in clinically typical cases of nephronophthisis suggests that needle biopsy may be a useful diagnostic means in patients with atypical clinical picture In good quality specimens, which include both cortex and medulla, the presence of tortuous, atrophic medullary tubules with thickened basement membranes and cysts in *the medulla* are *highly suggestive*

Medullary cysts have previously been *reported as a feature in the histological* picture of nephronophthisis [1 4 9], but the occurrence of cysts in the renal medulla has also been recognized as "medullary cystic disease [14] Whether this is an entity or not is obscure At any rate the limits of this entity are probably too wide and may include for example nephronophthisis.

The glomerular lesions are not very characteristic in nephronophthisis and it is difficult to distinguish them from those encountered in e.g chronic pyelonephritis. When compared with glomerulonephritis *the differentiation is more simple in that* not all glomeruli are affected and no semilunar crescents are encountered. In Table 4 we have listed some clinical and morphological differences between nephronophthisis, typical and atypical, and other diseases with which it may be confused.

Findings in a previous investigation of typical cases of nephronophthisis suggested that renal tubular damage preceeds glomerular degeneration (2) In the patients reported here water conservation seemed to be reduced more in parallell to glomerular filtration than in the typical cases and this resembles the situation in chronic

TABLE 4 *Comparison between nephronophthisis (typical and atypical) and other renal diseases appearing with one or several of the following signs heredity for renal disease pitressin resistant water losing syndrome reduced GFR with normal urine findings and arterial hypertension*

| Type of renal disease | Clinical dissimilarities t juvenile nephronophthisis | Morphological dissimilarities to juvenile nephronophthisis |
|---|---|---|
| Chronic glomerulonephritis (of different types) | No marked polydipsia. Renal history often positive. Proteinuria moderate—massive. Erythrocyturia. Cylindruria | Glomerular lesions generalized, often with some formation of semilunar crescent |
| Chronic pyelonephritis with or without renal parenchymal dysplasia | Renal history often positive Leucocyturia and/or bacteriuria appear at least intermittently | Kidneys often unequal in size with irregular outline. Patchy contraction of the parenchyma. Infiltration by inflammatory cells. Thyroid like changes of tubules. Dysplastic structures |
| Bilateral obstructive uropathy | Renal history often positive. Leucocyturia and/or bacteriuria appear at least intermittently Hydronephrosis. Obstruction of urinary pathways | Hydronephrotic trophy often with inflammation of renal parenchyma |
| Polycystic kidney disease ("adult type) | Often lumbar pains. At least intermittent haematuria. Often complicated by infection. Often typical -ray | Kidneys enlarged. Cyst formation in the entire kidney |
| Medullary sponge kidney | Renal colic common. Intermittent gross haematuria. Typical findings on urography | Lesion localized t inner medulla and papillary tip with inflammation and calculi |
| Primary renal acidosis | Inability to lower urinary pH much below 7.0. Nephrocalcinosis | Typical nephrocalcinosis |
| Primary pitressin resistant diabetes insipidus | Normal glomerular filtration after hydration. Kidneys of normal size | Kidneys histologically unremarkable |
| Primary hyperparathyroidism | High urinary calcium excretion. At least intermittently raised serum calcium levels. Nephrocalcinosis and hypertension in later stages | Nephrocalcinosis, often with inflammation of the renal parenchyma. Hypertensive vascular changes in the kidneys |
| Primary hyperaldosteronism | Typical electrolyt pattern in serum and urine | Often hypokalaemic nephrosis. Otherwise histologically normal kidneys |
| Balkan nephropathy [7]. | Typical geographical distribution. Children rarely affected, otherwise strikingly similar to nephronophthisis. | Characteristic irregularity in the distribution of the cortical trophy Often marked infiltration by inflammatory cells. Cysts, if present, are of the microcystic "thyroid like type. |

glomerulonephritis. The morphological examination of the two patients in this study gave no further information bout the sequence of events leading to the tubular and glomerular damage both tubules nd glomerulli being severely altered.

The etiological background of the clinical differences between the typical and atypical forms of the disease is obscure but may have a genetic explanation. Both recessively and dominantly transmitted diseases may take on certain features in one family and somewhat dissimilar ones in others But while recessively inherited

diseases usually show great similarities among siblings dominant ones may vary much in their expression even within one and the same family No conclusion about the mode of inheritance can be drawn from our present investigation, although the apperance of both typical and atypical disease in one family (Case 2) is noteworthy since nephronophthisis has been assumed to be inherited by a recessive, autosomal gene [12] The mode of inheritance is an unsolved question which is now under investigation and which gains some extra interest and importance from the fact that the disease may appear both in children and their parents or other adult relatives [10]


## Summary

Two patients with morphological and/or clinical and hereditary evidence of nephronophthisis are presented. They all lacked the most typical clinical symptom of the disease i.e. the water losing syndrome This "atypical" form of nephronophthisis is analysed and discussed in relation to "typical nephronophthisis and other chronic nephropathies. The mode of inheritance is briefly discussed.


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# Dietary Treatment of Protein Losing Enteropathy

by MINNA YSSING HERLUF JENSEN and STIG JARNUM

Severe hypoproteinemia due to enteric protein loss is reported with increasing frequency When present from early life it usually reflects extensive congenital malformations of the lymphatic system Typically the lesion produces a syndrome of asymmetrical oedema, chylous ascites and malabsorption with associated abnormal protein loss in the gut.

The treatment has remained palliative and often inefficient until a few years ago Recently however several successful therapeutic trials have been reported in both adult and paediatric cases in whom a strict dietary fat restriction was instituted [6, 7 15 21], or in whom medium chain triglycerides (MCT) were substituted for the ordinary triglycerides (LCT) of the food [5 16].

In the following we report on a milk fed infant who was subjected to a dietary treatment along these lines. Th course was complicated by the development of severe allergy to cow s milk proteins

## Case History

M. S., male infant born March 1965, was admitted to the paediatric department at Rigshospitalet, Copenhagen, 7½ weeks old, with a diagnosis of oedema. H was the second child of healthy parents. There was no family history of similar disorders. The pregnancy was uneventful. The birth was four weeks before term. The birthweight was 2300 g, the length 47 cm. There were no neonatal complications. The child was breast fed for the first two weeks. After that time cow milk was added. By the ge of three weeks breastfeeding was given up, artificial feeding continued with a diluted cow milk formula. N digestive disturbances were noticed during these first weeks of life.

By the age of 7 weeks peripheral oedema appeared, rapidly followed by facial oedema, and after a few days anasarca developed. On admission to our linic the general condition of the patient was poor H was pale with decreased muscle tone Facial and peripheral oedema was present, especially pronounced at the left lower extremity (Fig. 1). The abdomen was distended. The liver and spleen were not felt, and no ascites was detected. H presented a universal pityriasis like skin eruption. From four t eight loose watery stools were passed daily on a diet containing human milk supplemented with dried half-skim cow s milk formula with fat content of 1.5 per cent (Eledon). His weight was 3300 g and his length 53 cm.

*Laboratory studie* showed an anemia with a haemoglobin of 9 9 g per 100 ml, whit blood cell 10 440 per mm and a normal differential count. Later on eosinophilia developed with an eosinophil count about 1000–1500 per mm³ The erythrocyt sedi

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mentation rate was normal. The urine contained no sugar or protein, and the sediment was normal. Blood urea and serum creatinine were normal. The plasma sodium was 135 potassium 4 4, chloride 116 mEq/l, standard bicarbonate 18.5 mEq/l, serum calcium 8.2 mg per 100 ml, serum phosphorus 7 4 mg per 100 ml, and alkaline phosphatase in serum 21 King Armstrong units per 100 ml. Stool cultures were negative. A slightly elevated renal excretion of methionine and aspartic acid was found on one occasion. However repeated examinations failed to reveal any abnormal aminoaciduria. The total serum protein was 4.25 g per 100 ml with a serum albumin of 2.4 g per 100 ml, gradually decreasing to 1.2 g per 100 ml in spite of intravenous infusions of human albumin and dietetic extra protein supply. The serum gamma-globulin was within normal physiological range for the age. Alpha and beta globulins were normal, as was immunoelectrophoresis of serum. Serum triglycerides, serum cholesterol and serum phospholipids were within normal limits. Hepatic function was unimpaired as judged from a normal serum bilirubin, a normal thymol turbidity test in serum, a normal prothrombin time, and a normal concentration of glutamate pyruvate transaminase in serum. No evidence of renal disease was found. Intravenous pyelograms were normal. Electrocardiogram and radiogram of the heart were normal. Oral glucose-lactose- and saccharose loading gave a normal rise of the blood sugar concentration. A D-xylose test showed a subnormal excretion in the urine (14 of the oral dose). Faecal fat was slightly raised (17 % of an oral intake of 1 g butterfat daily). Radiological examination of the small intestine showed flocculation and segmentation of the barium meal and coarsening of the mucosal folds in jejunum and ileum, whereas the stomach and colon were found to be normal.

A tentative diagnosis was made of "idiopathic hypoproteinaemia with protein losing enteropathy. It was confirmed by investigations with labelled albumin and imferon (see Special Investigations).

## Special Investigations

### $^{59}$Fe-imferon test

The introduction of $^{59}$Fe-imferon to demonstrate gastrointestinal plasma protein loss is new [2].

Imferon is identical to iron-dextran. The molecular weight of the preparation is about 180,000. $^{59}$Fe is present in a very stable bond in imferon. Consequently no free $^{59}$Fe is found in the preparation.

Following intravenous injection of $^{59}$Fe-imferon to normal persons the plasma radioactivity declines very rapidly (T/2 about 0.5 days). No radioactivity appears in urine and less than 1 per cent of the injected dose in faeces. The major part is taken up by the reticulo-endothelial system. The advantage of $^{59}$Fe-imferon compared to $^{51}$Cr-albumin, often used to demonstrate gastrointestinal protein loss [17-20], is the lack of urinary excretion of $^{59}$Fe. Especially in infants and children the separation of urine and faeces may be difficult. Using $^{59}$Fe-imferon no false positive tests can result from urinary contamination of faeces.

In this laboratory a fair correlation between faecal excretion of $^{59}$Fe and $^{51}$Cr has been found in both controls and in patients suffering from gastrointestinal protein loss.

*Procedure.* About 0.5 μc $^{59}$Fe-imferon was injected intravenously and the faecal excretion of $^{59}$Fe in the next four days was measured.

Concerning blood samples, urine collection and measurement of radioactivity see infra.

### $^{131}$I-albumin turnover study

Albumin turnover study was performed with $^{131}$I labelled albumin. Physico-chemical and metabolic characteristics of the albumin preparation used are reported elsewhere [13].

*Procedure.* About 1 μc $^{131}$I-albumin was injected intravenously.

Blood samples were drawn in test tubes containing dried heparin, 15 minutes after

Obtained from Behring Werke Marburg, Lahn, Germany (Albumin- reinst ).

the injection and then once a day Urine and faeces were collected in 24 hour specimens.

The radioactivity in plasma and urine was measured in 1–3 ml aliquots, whereas the activity of faeces was measured in the whole 24 hour specimen.

The radioactivity was measured in an auto-gamma-spectrometer (Packard) and compared with that of a standard solution containing a known fraction of the injected dose

Total protein concentration (Biuret) was determined in all plasma samples in order t obtain specific activity (counts per minut per mg protein).

To prevent thyroid uptake of ¹³¹I liberated by catabolism of ¹³¹I-albumin the patient was given potassium iodide twice a day in an amount corresponding to 25 mg iodine daily

### *Parameters*

Serum albumin was determined by paper electrophoresis.

*Plasma volume* ($PV$) was calculated by di vision of the injected amount of radioactivity by the radioactivity in 1 ml plasma 15 minutes after the injection. The radioacti vity of the plasma sample was corrected with a factor 1 02 to allow for extravascular deposition of the labelled protein during the 15 minutes [4 11].

*Intravascular mass* ($IVM$) equals:

$$\frac{PV\ (\text{ml})}{100}\ \text{serum albumin (g/100 ml)}.$$

*Distribution ratio* ($D$) is the percentage of total albumin located intravascularly This parameter was calculated according t the method of Nosslin & Bryde Andersen [1].

$$\text{Total mass } (TM) \text{ equals } \frac{IVM \quad 100}{D}$$

*Fractional catabolic rat* ($FCR$) is the percentage of $IVM$ degraded per da both by catabolism and gastrointestinal loss This alue was obt ined by graphical resol tion of the plasma act vity curve according to the method given by M tthews [9].

*Synthetic rate* ($S$) (g dav or mg/kg/day). Assuming steady state the synthesis of albu min must equal the cat bolism f the protein. Consequently $S = (IVM \quad FCR)/100$ g/day

## Results

The patient was studied twice with ⁵⁹Fe imferon and ¹³¹I-albumin simultaneously The first study was performed in June 1965 when the patient was 3½ months old. During this investigation the ⁵⁹Fe-imferon test was abnormal. The faecal excretion of ⁵⁹Fe in 4 days after the injection amounted to 8 0% (normal <1 0) This figure is consistent with an abnormal gastrointestinal protein loss. The result agreed well with the simultaneously performed ¹³¹I-albumin turnover study Fractional catabolic rate was 40% per day Normal values of $FCR$ for infants will presumably be from 25 to 30% [8] The synthetic rate of serum albumin was normal. The results are summarized in Table 1 together with the results of a second study perfor med during treatment September 1965 (*vide infra*)

*Treatment and subseq ent course* (Fig 2)



Fig 1 Protein losing enteropathy with asymmetrical oedema. Crural oedema was more pronounced on he left side

TABLE I $^{131}I$ *Albumin degradation study and* $^{59}Fe$ *imferon test*

Diet during first investigation: Human milk + dried skimmed cow milk (Eledon). Diet during second investigation: Skimmed human milk + MCT

$Q_F$. Faecal excretion of the isotope in 4 days as per cent of injected dose. *PV* Plasma volume. *IVM* Intravascular mass of albumin. *TM* Total mass of albumin. $FCR_F$. Fractional catabolic rate of $^{131}$I-albumin, % of *IVM* per day *S* Synthetic rate. $MLT_{ws}$. Mean life time of an albumin molecule in the body

| | $^{131}I$-*albumin turnover* Weight (g) | $Q_F$ | Serum albumin (g/100 ml) | *PV* (ml) | *IVM* (g) | *TM* (g) | $FCR_F$ | *S* g/day | *S* g/kg/day | $MLT_{ws}$ | $^{59}$Fe-imferon test $Q_F$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| First investigation, June 1965 | 4090 | 2.4 | 1.48 | 190 | 2.8 | 5.0 | 40 | 1.2 | 0.29 | 4.5 | 8.0 |
| Second investigation, Sept. 1966 | 4500 | — | 2.50 | 210 | 5.3 | 13.7 | 28 | 1.5 | 0.33 | 9.3 | <1.5 |

By the age of 3½ months the child was put on a synthetic milk formula containing casein, lactose, electrolytes in appropriate concentrations and medium chain triglycerides. The diet was not tolerated. After a week the diarrhoeas exacerbated and a state of hypertonic dehydration resulted, which had to be corrected by parenteral fluid therapy. This course of events was later repeated on two occasions at 7½ and 8½ months of age where human milk was experimentally replaced by a cow's milk formula. At the last experiment the child started vomiting immediately after the meal, and a few hours later he passed watery stools containing fresh blood.

From the age of 4½ months the boy was put on a diet of skimmed human milk with medium chain triglycerides added in a concentration of 3.3 g per 100 g milk. The content of long chain triglycerides in the skimmed milk was found to be negligible (0.25 g per 100 ml) on repeated examinations. When the child was 6½ months old, the diet was supplemented with rice, vegetables with a low fat content, and boiled lean fish. The daily maximum intake of LCT fat was about 2.5 g. From the age of 9½ months the child was again given skimmed cow's milk in increasing amounts. After 4 weeks it was completely substituted for human milk. A daily supply of A, B, C, D and F vitamins was added to the diet.

The course before treatment was characterized by severe malnutrition with a varying degree of oedema and persistent diarrhoeas, anemia, and a low constantly falling serum albumin concentration. Motor retardation was prominent. By the age of 5 months the child was unable to hold his head. He was flaccid with decreased muscle tone and a persistent Moro reflex. The tendon reflexes were normal and no evidence of tetany was found. His mental development was estimated to be within normal limits considering his serious somatic illness.

After one week of treatment with skimmed human milk with MCT a clinical improvement was evident. His oedema

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Medium chain triglycerides (MCT) were kindly supplied by Drew Chemical Corporation, Boonton, New Jersey, U.S.A.

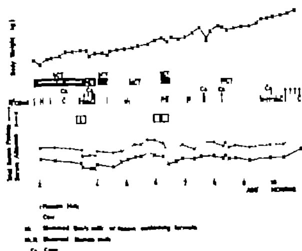


Fig. 2. Course and treatment in an infant with protein losing enteropathy [I] and [II] mark the two periods of investigation with $^{131}$I-albumin and $^{59}$Fe-imferon.

diminished and yet he gained in weight The daily number of stools decreased, and the cutaneous manifestations disappeared. However the oedema remained visible at the left lower leg and to a lesser degree in the right lower extremity After six weeks of dietary treatment the serum albumin level had increased from 1.2 g per 100 ml to a maximum value of 3.2 g per 100 ml. Later on a rather constant level of serum albumin concentration between _0 and 2.5 g per 100 ml was found. The total serum protein concentration reflected the albumin concentration.

The anemia was successfully corrected by oral iron administration. Faecal f t was normalized after months of treat ment. (F lling to 6% of an oral intake of _5 g butterfat (LCT) and 3* g MCT daily). Normal values for serum calcium (9.3 mg per 100 ml) nd serum phosphorus (4 1 mg per 100 ml) were obtained at the same time while the serum concentration of alkaline phosphatase was found in the upper normal range (27 King Armstrong units per 100 ml) No clinical signs of rickets were evident at any time, and no rachitic changes were noticed by radiological examination of the wrist when the child was 7 months old. No intercurrent infections complicated the course His motor development improved gradually his growth enhanced, but remained retar ded.

A second study with $^{59}$Fe-imferon and $^{131}$I-albumin was performed after 6 weeks of treatment with skimmed human milk and medium chain triglycerides.

Now the $^{59}$Fe imferon test was normal. Fractional catabolic rat for albumin amounted to 28 per cent Serum albumin increased from 1.5 to _5 g per 100 mg between the two studies (Table 1)

In order to demonstrat the significance of long chain triglycerides in provoking the protein loss in th gut the patient was

TABLE I $^{131}I$ *Albumin degradation study and* $^{59}Fe$ *imferon test*

Diet during first investigation: Human milk + dried skimmed cow milk (Eledon). Diet during second investigation: Skimmed human milk + MCT

$Q_F$. Faecal excretion of the isotope in 4 days as per cent of injected dose. *PV* Plasma volume. *IVM* Intravascular mass of albumin. *TM* Total mass of albumin. $FCR_p$. Fractional catabolic rate of $^{131}$I-albumin, % of *IVM* per day *S* Synthetic rate. $MLT_{WB}$ Mean life time of an albumin molecule in the body

| | $^{131}I$-albumin turnover Weight (g) | $Q_F$ | Serum albumin (g/100 ml) | *PV* (ml) | *IVM* (g) | *TM* (g) | $FCR_p$ | *S* g/day | g/kg/day | $MLT_{WB}$ | $^{59}Fe$-imferon test $Q_F$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| First investigation, June 1965 | 4030 | 2.4 | 1.48 | 190 | 2.8 | 5.0 | 40 | 1.2 | 0.29 | 4.5 | 8.0 |
| Second investigation, Sept. 1965 | 4600 | — | 2.60 | 210 | 5.3 | 13.7 | 25 | 1.5 | 0.33 | 9.3 | < 1.5 |

By the age of 3½ months the child was put on a synthetic milk formula containing casein, lactose electrolytes in appropriate concentrations and medium chain triglycerides. The diet was not tolerated. After a week the diarrhoeas exacerbated and a state of hypertonic dehydration resulted, which had to be corrected by parenteral fluid therapy This course of events was later repeated on two occasions at 7½ and 8½ months of age where human milk was experimentally replaced by a cow's milk formula At the last experiment the child started vomiting immediately after the meal, and a few hours later he passed watery stools containing fresh blood.

From the age of 4½ months the boy was put on a diet of skimmed human milk with medium chain triglycerides added in a concentration of 3.3 g per 100 g milk. The content of long chain triglycerides in the skimmed milk was found to be negligible (0.25 g per 100 ml) on repeated examinations When the child was 6½ months old, the diet was supplemented with rice, vegetables with a low fat content, and boiled lean fish. The daily maximum intake of LCT fat was about 2.5 g From the age of 9½ months the child was again given skimmed cow's milk in increasing amounts. After 4 weeks it was completely substituted for human milk A daily supply of A, B C D and E vitamins was added to the diet

The course before treatment was characterized by severe malnutrition with a varying degree of oedema and persistent diarrhoeas, anemia, and a low constantly falling serum albumin concentration. Motor retardation was prominent By the age of 5 months the child was unable to hold his head. He was flaccid with decreased muscle tone and a persistent Moro reflex. The tendon reflexes were normal, and no evidence of tetany was found. His mental development was estimated to be within normal limits considering his serious somatic illness.

After one week of treatment with skimmed human milk with MCT a clinical improvement was evident His oedema

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Medium chain triglycerides (MCT) were kindly supplied by Drew Chemical Corporation, Boonton, New Jersey U.S.A.

sent in this case He was not particularly susceptible to infections, and serum con tained a normal amount of isoaggluti nin.

Idiopathic coeliac disease seemed to be less probable because of the early debut of the disease at a time where no gluten was included in the diet

The etiology of the underlying intestinal disease was probably congenital malforma tion of the intestinal lymphatic apparatus. The asymmetrical oedema of the lower extremities was evidence of more wide-spread lymphatic disease [19]. A small intestinal biopsy would be able to confirm the diagnosis by the demonstration of intestinal lymphangiectasia" However we refrained from this diagnostic procedure due to the infant s age and the well documented risk of small intestinal biopsies in this age group [10 14]

Concerning the long term prognosis of intestinal lymphangiectasia subjected to low fat diet with MCT one must await further experience. So far the present case has been followed 8 months on MCT treatment. The growth is still retarded but the mental development seems to be entirely normal. Although serum albumin concentration rose a normal serum protein concentration was not achieved.

In a 5-year-old child with intestinal lymphangiectasia followed through _ years on a MCT formula a normal growth was initiated when diet was instituted [16]. We have observed a marked and still existing remission on this treatment in a 20-year old man with stunted growth emaciation and generalized oedema with hypoproteinemia almost since birth [6]. H had now been f llowed for 10 months.

Despite the clinical improvement and the increase of serum albumin, none of these cases obtained a normal serum protein concentration It is well compatible with the conception that MCT treatment is a purely palliative though efficient treatment of protein-losing enteropathy

The cause of the beneficial effect of MCT treatment is not quite clear In vestigations by Borgström & Laurell [3] have shown that the lymphatic protein flow from the intestine increases during absorption of fat (LCT). It implies that fat (LCT) increases the transfer of serum protein across the blood capillaries of the intestinal mucosa. Consequently a lower transcapillary protein transfer t kes place during a low fat diet, which could be part of the explanation of a successful treatment with this diet in protein losing enteropathy

MCT is absorbed directly to the blood stream in contrast to LCT which is absorbed to the lymphatic system as chylomicrons. MCT *pe se* has hardly any effect on the abnormal protein loss, but, being without influence on the lymph flow it is highly valuable as a calorigenic supplement to a low fat diet.


## Summary

A case of protein losing enteropathy in an infant is presented Peripheral oedema ppeared 7 weeks after birth A hyper cat bolic hypoalbuminaemia was found with $^{131}$I-albumin, and abnormal gastrointestinal prot in loss was detected by means of $^{59}$F imferon Because of asym metrical oedema which ndicated lym phatic malformatron t is considered that intest nal lymphangiectasia" underlay the prot in loss.

A low fat diet based on skimmed human milk supplemented with medium chain triglycerides (MCT) resulted in a definite improvement. Serum protein rose to subnormal levels oedema diminished and growth was enhanced.

The course was complicated by severe allergy to cow milk s proteins, apparently unrelated to the protein-losing enteropathy When the child was 10 months old hyposensibilization was successfully carried out by gradual substitution of skimmed cow s milk for skimmed human milk.

## Addendum

Following the submission f the paper the child was dismissed to his home. Five days later he suddenly began to pass large amounts of watery diarrhea. He was readmitted in a state of dehydration shock and despite intensive treatment he succumbed within less than 24 hours. Autopsy (Dr Inge Tygstrup) showed numerous dilated lymphatics in the small intestinal submucosa and in the villi. The hanges were most pronounced in the jejunum. The remaining part f the gastrointestinal tract was normal. Kidneys, liver pancreas and thoracic lymph nodes were all normal. In the mesentery only very few and hypoplastic lymph nodes were present. The thoracic duct was not identified.

Thus, although no explanation was found of the acute episode leading to the patient death, the autopsy confirmed the presumed diagnosis of intestinal lymphangiectasia. It is probable that it was due to congenital hypoplasia of mesenteric lymphatics and lymph nodes.

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# Effect of Physical Training on School Children with Cerebral Palsy

*by* ÅKE LUNDBERG CARL-OLOF OVENFORS and BENGT SALTIN[1]

There is a shortage of data concerning the physical working capacity of young persons suffering from cerebral palsy (CP) and the degree to which the oxygen transport capacity of the respiratory and circulatory system can be increased of the individual.

Children and young persons with CP run the risk of becoming inactive as a result of their motor disturbances. This risk has been considered most serious also in non-motor-disturbed handicapped groups, for blind patients by Mossfeldt & Sjöstrand, 1960 [11], and in chronic mentally-ill patients, by Carlsson *et al.*, 1965 [5], who reported a favourable development of the patients physical capacity following systematic physical training

A group of school-children with CP underwent systematic training in order to increase their physical working capacity To measure the results of this training heart volume oxygen uptake heart rate and blood lactic acid were determined during submaximal work before and after training

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Research Fellow on grant from the Insurance Company Förenade Liv Stockholm.

## Material

The material (Table 1) consisted of 14 students from the Norrbacka Institut schools—4 girls and 10 boys. Ages ranged from 15–20 years, with a median age of 17½ years. Five of these students were in help-classes, the rest in regular classes. The diagnoses included 3 cases of hemiplegia, 3 of diplegia and 3 of tetraplegia. 4 patients had athetosis and 1 patient had ataxia.

Degrees of motor disturbance ranged from slight to fairly serious. The school-children were selected for this investigation on the basis of their ability to operat the bicycle ergometer

All the student fell with ±2 standard deviations in the growth diagram (Table 2) of Karlberg & Iggbom [8].

## *Methods*

Before training the condition of ll the students was checked by an ordinary physical examination and ECG All had experiences of cycling before the first tests were carried out though some had to have their feet strapped to the pedals.

Physical training was carried out in 30-minut periods twice a week for six weeks during March–April, 1965, concurrently with the usual school gymnastic lessons which totalled 3 hours a week. This particular period in early spring was selected in order to exclude the influence of the regular school gymnastics on bodies that are in a relatively

TABLE 1 *Material*

| Case No. | | Sex | Age | Class | Cerebral palsy |
|---|---|---|---|---|---|
| 1 | A. L. | F | 16 | Regular | Hemiplegia |
| 2 | A. L. | F | 18 | Regular | Tetraplegia |
| 3 | J. E. | F | 16 | Regular | Diplegia |
| 4 | W. Y. | F | 19 | Help-class | Athetosis |
| 1 | B. S. | M | 17 | Regular | Diplegia |
| 2 | B. O. | M | 18 | Regular | Athetosis |
| 3 | G. A. | M | 16 | Regular | Athetosis |
| 4 | H. H. | M. | 19 | Regular | Hemiplegia |
| 5 | I. B. | M | 17 | Help-class | Tetraplegia |
| 6 | J. S. | M | 18 | Help-class | Athetosis |
| | K. Y. | M | 16 | Help-class | Diplegia |
| 8 | L. G. | M | 15 | Regular | Tetraplegia |
| 9 | Y. C. | M | [illegible] | Regular | Ataxia |
| 10 | O. Y. | M | 15 | Help-class | Hemiplegia |

untrained condition after the summer holidays. The training was devised to exercise large muscle groups continously for at least 1 2 minutes, and therefore consisted f running, jumping using parallel bars and so on, as much as their physical handicap permitted. In the pauses between the various exercise shifts, the students kept jogging t avoid inactivity In one boy (No. 1) heart rat was determined during certain parts of the training programme and varied between 158–184 beats per minute.

Attendance at the training sessions was, on the verage, 11 times per patient out of 13 possible times, varying from 7 times (girl No. 2) t 13 times (boys Nos. 1 5, 8, 10).

Roentgenological heart volume determination and identical work tests were carried out before and after the training period.

Heart volumes were determined according to Larsson & Kjellberg [10], with minor modifications. Roentgenograms were taken with the patient recumbent while resting and breathing normally The vertical and horizontal rays were so directed that the antero-posterior and lateral projection were obtained.

TABLE 2. *Height nd weight.*

| | Length, cm | | Weight, kg | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| Girls | 162 | 153–171 | 53 | 40–67 |
| Boys | 173 | 164–185 | 60 | 51–65 |

The films were exposed one at a time irrespective of cardiac cycle or respiratory phase Focus-film distance was 125 cm and the enlargement of the roentgen picture was determined by means of a centimeter scale at the film level. Heart olume was deter mined by means of Kahlstorf formula for an ellipsoid [7] but using the modifications introduced by Larsson & Kjellberg [10].

The work tests was carried out on a mechanically-braked bicycle ergometer [8] with pedal rate of 50 rpm, if possible.

The girls started t 300 kpm/min work intensity and the boys t 600 kpm/min, for 6 minutes. Some hildren were immediately afterwards capable of heavier work—that is, 450–600 kpm/min for the girls and 600–900 kpm/min for the boys. None of the girls could perform what might be called maximal work, but four boys (Nos. 1 4 6, 8) achieved maximal work. During the maximal work period, tests for heart rat and oxygen uptake became adjusted to the work period achieved.

Three boys (Nos. 3 6 and 10) had no dif ficulty in working the bicycle ergometer and therefore oxygen uptake at 600 kpm/min was not determined.

Oxygen uptake was determined by the Douglas bag method, and gas analyses were carried out by Haldane method. Maximal oxygen uptake was determined with Åst rand normogram [1].

Heart rat was calculated from recordings taken t one minute intervals during bicycle work with one-channel Siemens ECG machine Comparable values were taken as means from the 5th and 6th minutes during the exercise tolerance test

Lactic acid concentration in the blood was determined by the Barker-Summerson method, modified by Ström [13]. T obtain the highest value –3 test were taken f llowing each work-load.

## Result

### *Females*

The 4 girls all proved capable of 300 kpm/min. The mean value for oxygen uptake was 1 00 l/min *before training*, and 0 99 l/min after training (Table 3 Fig 1) The heart rate at this work load was 150 beats and 142 beats, respectively (Table 3 Fig 2) Lactic acid concentration in the blood was 23 mg per 100 ml at 300 kpm/min before training and dropped to 16 mg per 100 ml after training (Table 3 Fig 3)

The mean value of oxygen uptake with the heavier work load (mean = 550 kpm/min) which 3 of the girls (Nos. 1 ? and 4) carried out was 1.34 and 1.32 l/min before and after training respectively (Table 3 Fig 1) Heart rate was 172 beats before training as against 164 after (Table 3 Fig 2) The blood lactic acid concentration showed the same trend, being 57 mg per 100 ml before and 49 mg per 100 ml after training (Table 3 Fig 3)

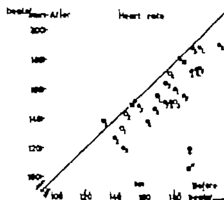



Fig. 2. Heart rate during submaximal work-load before and after 6-week training period. (For symbols, see Fig. 1 )

The calculated maximal oxygen uptake increased during training for 3 of the 4 girls, 0.25/min on the average (Table 4 Fig 4) The girl (No 4) with the unchanged value at maximal oxygen uptake had the highest value even before training

Heart volume could be measured for only two of the girls (Nos. 1 and ) before and after training Girl No 1 increased from 470 to 570 ml, while girl No 2 increased only from 600 to 610 ml (Fig 5).

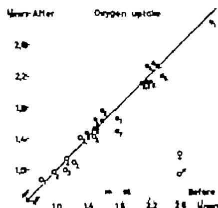



Fig 1 Oxygen uptake during submaximal work load before and after 6-week training period. (The values before training are given along the X-axis, the values after training along the Y axis. The line of identity is marked. The figures beside the symbols give the patient number in the material. The point after the figure shows that the value refers to the higher of the two submaximal work-loads.)

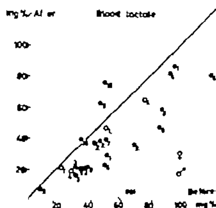



Fig. 3. Blood lactat during submaximal work load before and after a 6-week training period. (For symbols, see Fig. 1 )

TABLE 3 *Results*

B = before training; A = after training; $n$ = number of observations, $\bar{x}$ = mean of individual observations; $R$ = range; $p$ gives the degree of significance for the difference between B and A for girls and boys.

| | Submaximal load I | | | | | | | Submaximal load II | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Oxygen uptake | | Heart rate | | Blood lactate | | | Oxygen uptake | | Heart rate | | Blood lactate | | Heart volume | |
| | Kpm/min | B | A | B | A | B | A | Kpm/min | B | A | B | A | B | A | B | A |
| ♀ $n$ | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 |
| $\bar{x}$ | 300 | 1.00 | 0.99 | 150.3 | 141.8 | 23.0 | 16.0 | 580 | 1.34 | 1.32 | 171.7 | 164.3 | 57.3 | 48.7 | 535 | 590 |
| $R$ | 300 | 0.90– | 0.89– | 133– | 133– | 9– | 8– | 450– | 1.22– | 1.09– | 167– | 156– | 44– | 30– | 470– | 570– |
| | 300 | 1.13 | 1.14 | 170 | 152 | 32 | 21 | 600 | 1.50 | 1.44 | 176 | 172 | 77 | 64 | 600 | 610 |
| ♂ $n$ | 10 | 7 | 7 | 10 | 10 | 10 | 10 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 10 | 10 |
| $\bar{x}$ | 600 | 1.59 | 1.58 | 166.8 | 150.9 | 46.9 | 32.9 | 964 | 2.29 | 2.29 | 188.9 | 170.9 | 80.7 | 65.0 | 668 | 695 |
| $R$ | 600– | 1.29– | 1.44– | 130– | 120– | 29– | 20– | 900– | 1.99– | 2.02– | 166– | 133– | 48– | 35– | 490– | 630– |
| | 600 | 1.77 | 1.73 | 196 | 189 | 68 | 63 | 1200 | 2.96 | 2.80 | 209 | 191 | 134 | 92 | 820 | 775 |
| $p$ | | > 0.05 | | < 0.001 | | < 0.001 | | | > 0.03 | | < 0.001 | | < 0.001 | | > 0.1 | |

*Males*

The 10 boys were all able to do 600 kpm/min. Oxygen uptake with this work load was, on the average 1.59 and 1.58 l/min (7 boys) before and after training, respectively (Table 3 Fig 1) The heart rate with this work load dropped, as a result of training, from 167 beats/min to 151 beats/min (Table 3, Fig. ). This was likewise true of lactic acid content in the blood which before training at 600 kpm/min was 47 mg per 100 ml and after training 33 mg per 100 ml (Table 3 Fig 3).

TABLE 4. *Calculated maximal oxygen uptake capacity*

| | | Before training | | After training | |
|---|---|---|---|---|---|
| | | l/min | ml/kg min | l/min | ml/kg min |
| Girls $\bar{x}$ $V_{O_2}$ | 4 | 1.65 | 29 | 1.80 | 34 |
| Boys $\bar{x}$ $V_{O_2}$ | 10 | [illegible].25 | 39 | 2.60 | 44 |

With the higher work load (mean = 964 kpm/min) carried out by 7 male students the oxygen uptake was, on the average, [illegible].29 l/min both before and after exercise (Table 3 Fig 1). The heart rate dropped, as a result of training on this work load, by 18 beats/min from 189 to 171 (Table 3 Fig 2) and the blood lactic acid concen

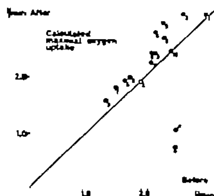



Fig 4. Calculated maximal oxygen uptake capacity before and after 6-week training period. (For symbols, see Fig. 1)

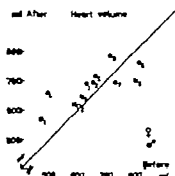



Fig. 5. Heart volume before and after a 6-week training period. (For symbols, see Fig 1)

tration from 81 to 65 mg per 100 ml (Table 3 Fig 3)

The calculated maximal oxygen uptake increased for 9 of the boys and remained unchanged for the tenth (No. 10) The average increase was 0.35 l/min (Table 4 Fig 4), with a variation from 0 to 1.60 l/min.

Heart volume after training was, in 7 cases, *0 to 170 (mean = 67) ml greater and in the remaining 3 cases (Nos 5, 6 7) 40 to 100 (mean = 67) ml less. On the average it increased *7 ml/male patient (Table 3 Fig 5)

## Discussion

The aim was to determine maximal oxygen uptake in a group of students with cerebral palsy before and after a 6-week training period On account of movement handicaps and, in certain cases, fear and unfamiliarity with hard work, it was impossible to obtain maximal determinations, and therefore the changes in aerobic work capacity were judged from submaximal work test

The two chief sources of error in calculating maximal oxygen uptake by Åstrand's normogram [1] are

1 Individual variations in oxygen uptake at a certain workload.

2. Individual variations in maximal pulse rates.

*Ad* 1 When oxygen uptake is determined in all cases except one (boy No. 3) at one of the sub-maximal work-loads the first factor may be excluded as a source of error

*Ad* 2. Admittedly maximal heart rates were determined in only 4 cases (boys Nos. 1 4 6 8) but as these rates normally do not change during a brief exercise period [4], changes in maximal oxygen uptake can be calculated with considerable accuracy Less certain are the individual values for maximal oxygen uptake.

The calculated increase of 0.25 l/min for girls and 0.36 l/min for boys (Table 4, Fig 4) agrees with the findings from exercise studies on healthy young persons [4 9 1?] Neither in this nor in earlier investigations was there any possibility of reporting the intensity or the volume of the training programme carried out. It seems likely that this group of young CP patients exercised less absolutely than the exercise groups mentioned above That the improvement is equal or bigger can be explained by the fact that our group initially had a lower startinglevel as regards aerobic work capacity

With the heaviest work load before the start of the training period the boys (7 persons) had an average of 189 heart beats per minute with blood lactic ed concentration 81 per 100 ml. This fact together with the average age of 17 indicates a maximal heart rate of *00/*0, beats per minute Using this heart rate for calculation of the maximal oxygen uptake we obtain 1.55 (1.3–1.90) and 2.25 (1 70–

2 98) l/min for girls and boys, respectively before training. Expressed per kilogram of body weight the figures are 29 and 38 ml/kg and minute respectively (Table 4)

Compared with the normal values of Åstrand & Christensen [3] for corresponding age groups, the respective values for the girls and the boys are 25 % and 40 % lower After training period both the girls and the boys in the CP-group reduced the difference by an average of 10–15 %. This means that the boys in the CP-group are only 10 %, while the girls are still 25 % below the normal value for healthy young persons of corresponding age.

Determination of the roentgenological heart volumes with the patient lying down eliminates orthostatic factors. Exposure during unrestricted breathing excludes the influence of Valsalva effects.

As the exposures were not connected to ECG or respiratory recorders, the same phase in systole and diastole respectively cannot be btained. This circumstance taken together with the prevalence of thorax deformities in these children, considerably increases the sources of error and may be estimated at about ±8 %, which should be taken into ccount in measuring the non-significant changes in roentgenological heart volume before and after the exercise period.

During training, w have watched out for possible muscular complications. However in no case were w ble to find that exercise had a deleterious effect

Attendance varied somewhat, but this difference like the difference in improvement wa insufficient for any connection t be established.

A question of practical importance is how large a proportion of the student with cerebral palsy can be expected to carry out an exercise programme of this type The 14 patients described above constitute 25 % of the total of young CP patients at Norrbacka Institute and this would probably be representative of the percentage of students with CP able to participate in such an exercise programme The most severely handicapped children can hardly be expected at present to increase their physical performance but for that group of CP-children (about a further 25 %) whose motor disturbance consists principally of diplegia with inability to use the lower extremities, a bicycle ergometer has been constructed designed to be operated only by the arm and back muscles, so that the corresponding tests can be carried out after a training programme specially devised to strengthen the muscles in the upper half of the body

It is important to take advantage of opportunities for physical training in early youth when it is probably easier to develop the oxygen transport system than later in life [ ].

Summary

Fourteen students aged 15–20 with cerebral palsy carried out physical training programme lasting 30 minutes twice a week for six weeks concurrently with the ordinary school gymnastics. This training, which wa designed to exercise large groups of muscles, consisted of running jumping arm raising, etc. so far a th motor disturbance permitted.

Submaximal work tests on the bicycle rgometer were carried out before and ft the training period, with measurement of oxygen uptake pulse rate and blood lact ed Heart volume wa de

termined roentgenologically before and after the training period.

Heart rate was on the average, 8 and 17 beats per minute lower for girls and boys, respectively on the submaximal work loads after training and the blood lactic acid concentration was 8 and 14 mg per 100 ml lower respectively. The heart volume had increased on the average 30 ml (5 %).

The calculated values for maximal oxygen uptake capacity shows that the girls on the average increased their maximal oxygen uptake by 0.25 and the boys 0.35 l/min during training.

The results show that these young patients could easily increase their aerobic capacity by physical training. The conventional type of gymnastic training performed by school-children with *motor disturbances* should therefore be changed to *include more specific training* to improve their stamina and fitness. We consider that such training should be done concurrently with other therapy for the CP children.

## Acknowledgement

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# Investigation of a Family with Members with Both Severe and Mild Degree of Thrombasthenia

by STIG CRONBERG INGA MARIE NILSSON and ERIC ZETTERQVIST

Thrombasthenia is generally characterized by an increased bleeding tendency and prolonged bleeding time due to a qualitative platelet defect with consequent impairment of clot retraction and of th ability of platelets to adhere to glass and to aggregate [7 2] The term thrombasthenia was introduced by Glanzmann [6] who described patients with an increased tendency to bleeding and impaired clot retraction despite a normal number of platelets. Formerly before it was known that the AHF (f VIII) is decreased in von Willebrand's disease patients with this disorder were often misdiagnosed as having thrombasthenia In recent years patients with typical severe thrombasthenia have been found to have platelets that totally lack the ability to adhere to glass or to aggregate after addition of ADP thrombin or other stimuli. These characteristics clearly distinguish this condition from other types of haemorrhagic disorders and are usually considered obligatory for the diagnosis of thrombasthenia ].

This severe thrombasthenia is rare and most of the cases on record have been sporadic Occasionally however it has been seen in siblings or double cousins [1., 13 5 22] In a few of the cases on record the parents of the affected patients were related by blood [1., 20] A recessive mode of inheritance of this disease has therefore been assumed. But, as a rule no affected relatives could be traced. Marx & Jean [13], however reported one family where two sisters were severely ill, besides which one brother and the father had mild bleeding symptoms and a somewhat prolonged bleeding time A moderately pathological clot retraction in one parent was the only abnormality Hardisty *et al* [7] found in the relatives of one of their families.

We have recently had the opportunity of examining a large family in which two sisters and one of their female cousins had severe thrombasthenia. The parents and certain other relatives had mild bleeding disorders. The results are described below

## Methods

*Coagulation studies* Methods described in previous papers [14 15 16, 17] were used for collection of blood and for determination of the various coagulation factors and the components of the fibrinolytic system.

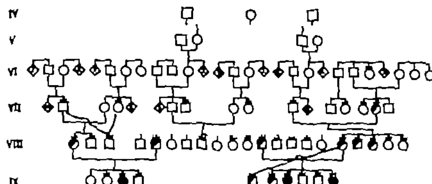



Fig 1 Pedigree of the family Symbols used in the pedigree: □ male; ○ female; ● female with severe thrombasthenia; ◧ ◐ persons with signs of mild haemorrhagic disorder (mild thrombasthenia); × above sex symbol indicates that the individual has been investigated; ◇ descendants of minor interest; VIII.5 = generation VIII, member no. 5 as counted from the left, descendants designated ◇ are not counted

*Platelet counts* were performed by Hellem modification [8] of Nygaard [19] method but using 3.8% sodium citrate. Control counts were also made by the method of Björkman [1].

*Platelet suspensions* were prepared and tested for platelet f ctors 1 3 and 4 as described previously [18]

*Platelet-rich plasma* was prepared by centrifuging citrated blood (1 part 3.8% sodium citrat and 9 part blood) drawn by the silicon technique at 185 g for 10 minutes immediately after collection.

*Platelet-deficient plasma* was prepared by centrifuging platelet-rich citrated plasma at 16,000 g for 30 minutes in plastic tubes at +4°C and then carefully pipetting off the supernatant plasma without disturbing the layer f platelets.

*Platelet adhesiveness* was measured according to a slight modification [3] of Hellem method [8] According to this method, citrated blood is passed through a column of glass beads and the percentage f adherent platelets is calculated Platelet adhesiveness in platelet rich plasma after addition of ADP in various concentrations was determined according t a slight modification [3] of the method of Hellem *et al.* [9]

*Aggregation f platelets t collagen f bres* Human fascia was homogenized About 0.5 g of the tissue was suspended in 10 ml f 0 9 saline. The solution was then centrifuged at 2000 g for 15 minutes. The slightly opalescent supernatant was used

*Clot retraction.* This was determined by a modification f Voss [23] method. 1 ml of platelet-rich plasma was diluted with 9 ml f 0 9% saline and coagulated with 0 1 ml of thrombin (T postasin Roche ) solution containing 200 N.I.H. units per ml. After some minutes, by which the clot had become firm, t was detached by rotating the tube. The length f the clot was measured immediately and then again after intervals of 3 and 24 hours. Clot retraction is expressed as a percentage calculated according to the f llowing form la:

$$\text{Clot retraction} = \frac{\text{original length of clot} - \text{length of clot after retraction}}{\text{original length of clot}} \times 100$$

Normal mean values are 70% after 3 hours and 79% after 24 hours.

## Clinical Material

The pedigree of the family in estigated is given in Fig. 1 Two sisters and one female cousin were found t be severely affected.

*Case 1* (IX.3)

A 19-year-old girl. Even in infancy she had bruised readily especially when creeping about on the floor At 7 years of age she was admitted to hospital because of haemarthrosis of the left knee On admission she had numerous petechiae. The bleeding time was prolonged, but the platelet count was normal, and blood transfusions were given. The following years she often had epistaxis necessitating cauterization. Gingival bleeding was also common. The most troublesome symptom now is her extremely profuse menstruations. In 1963 she was admitted to the department of gynaecology because of 2 months severe menorrhagia and was given several blood transfusions. She is continually receiving iron therapy

*Case 2* (IX.7)

A 7 year-old girl, a cousin of case 1 (the fathers were brothers) The patient had bruised readily already at 4 months of age. At 6 months she had a large haematoma after triple vaccination. She was then admitted to hospital, where the bleeding time was found t exceed 1 hour At 15 months she bled copiously after a gingival wound and was admitted to hospital, where the haemoglobin was found to be 8.8 g/100 ml. At 20 months she had severe nose bleeding and then the haemoglobin fell to about 5 6 g/100 ml. She was treated with blood transfusions. At 2½ years she again had severe epistaxis and on admission the haemoglobin was 5.9 g/100 ml. One week later nose bleeding recurred and the patient was again treated with blood transfusions. At 4 years of age she had a relapse and this time the haemoglobin fell t 4 g/100 ml. At 7 years aspiration of secretion in association with anaesthesia for dental treatment was complicated by severe nose bleeding

*Case 3* (IX 10)

A ear-old girl, sister of case 2. At 6 months of age the patient fell from chair and developed large haematoma in the scalp. Examination revealed Duke bleeding t me of more than 15 minutes. Petechiae were seen on the legs. At one year she had severe nose bleeding At 18 months she fell and hit her head and, as before a large haematoma developed in the scalp. The haematoma was evacuated, but soon recurred. She was treated with blood transfusions.

In none of these three patients was thrombocytopenia ever noted.

Five of the relatives reported a tendency to nose bleeding (IX:4, IX:5, VIII:12 VIII:18, VII 4); 4 copious menstrual bleedings (VIII:19 VII 7 VII:10 VII:12); the father of case 1 had had severe bleeding after tooth extraction (VIII:5), and the mother of the cases 2 and 3 had bruised readily (VIII:17)

## Coagulation Studies

All three severely ill patients had a markedly prolonged bleeding time of more than 30 minutes, when determined by the method of Duke (Table 1). On the other hand, the coagulation time was normal. Prothrombin consumption and thromboplastin generation were normal as were the concentrations of factors V VIII, IX, the prothrombin group (P&P) and fibrinogen. The one stage prothrombin time and the Stypven time were normal. There was no fibrinolysis.

*Platelets* At examination in the phase contrast microscope the platelets were of normal number size and morphological ppearance.

*Adhesiveness of platelets* Th platelets did not adhere to glass beads in tests performed according to Hellem [6] with citrated whole blood (Table 1) A similar lack of adhesion of platelets was noted when native blood immediately after withdrawal or heparinized blood (50 IU/ml, Vitrum ) was passed through the column w th glass beads.

In platelet-rich plasma platelets do not

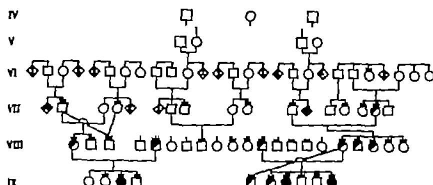



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into a drop of platelet rich plasma placed between a glass slide and a cover-slip Normally the platelets then first aggregate, immediately after which fibrin is precipitated and the platelet gradually undergo viscous metamorphosis. But when the test was carried out on the patients plasma the platelets did not aggregate Later they developed processes and were surrounded by a plasma zone and the granulomeres gradually dissolved. But they remained discrete The changes were thus essentially those seen in ordinary viscous metamorphosis, but without the initial aggregation.

On recalcification of platelet rich plasma from the patients the platelets behaved in the same way as seen after addition of thrombin.

*Clot retraction* Clot retraction of samples from the patients was not absent but slower and less complete than that of samples from normal controls with a corresponding platelet count (Table 1)

Addition of platelet free plasma from a normal person to platelet rich plasma from case 1 had no effect on the retarded clot retraction. The clot retraction of normal platelet rich plasma was not inhibited by addition of platelet-free plasm of the patient It would thus appear that the defective lot retraction f llowed the platelet and not the plasma.

## Family Investigation

As already stated in the description of the clinical material several relatives of the severely ill patients had more or less obvious symptoms of a haemorrhagic diathesis. Investigation revealed that in many of them the Ivy bleeding tim was prolonged nd the platelet dhesiveness decreased (Table ) Both of the mothers thus showed prolonged Ivy bleeding time (>30 minutes) and decreased platelet adhesiveness (16–?0 %) The mother of the cases * and 3 (VIII.1?) was examined thoroughly on repeated occasions. No other abnormality was found The clot retraction was normal (Table 1)

Of the fathers (VIII.5 and VIII 1.) who were brothers, the bleeding time was severely prolonged in one and moderately so in the other A prolonged bleeding time and a moderately decreased platelet adhesiveness were also observed in the maternal grandmother (VII 12) of the cases and 3 and in at least one of their siblings (IX.5) as well as in one of the mother s siblings (VIII 19) An Ivy bleed ing time of more than 30 minutes was also noted in one of the ther siblings of the patient (IX:6) and a normal bleeding time but decreased platelet adhesiveness in IX:9 and VIII 18 The bleeding time and platelet dhesiveness were normal in all of the father s siblings xamined.

Consanguinity was traced in the sixth generation between the parents of the cases 2 and 3, but not between the parents of case 1 (Fig 1). We were able to trace almost all ancestors in the first six generations, and most of those that were still living in the same district could be traced back through 8 generations and in a few cases through 9 generations—to the end of the seventeenth century

## Therapeut Trials

*Exercise* ha been described as increasing the content of factor VIII nd as shorten ing the bleeding time in patients w th von Willebrand disease [4 10] Case 1 was therefore xamined after cycling exercise

in very early generations or the registered parents might not always have been identical with the true or biological parents.

We have recently found 28 patients from 16 Swedish families with a mild haemorrhagic disorder characterized by prolonged Ivy bleeding time and moderately decreased platelet adhesiveness. In several of these families a dominant mode of inheritance could be demonstrated. The *mild haemorrhagic disorder* observed in these patients resembles that found in the relatives of the severely affected patients (cases 1, 2 and 3). The patients with the mild haemorrhagic disorder may be carriers of the gene for severe thrombasthenia but in a single dose. As both types have some features in common and are probably genetically interlinked, we would suggest the term "severe thrombasthenia" for the classical severe type with complete inability of the platelets to aggregate, and the term "mild thrombasthenia" for the mild cases.

That most investigators have not been able to demonstrate any abnormality in the relatives might be due to the expressivity varying from case to case and perhaps from time to time, to the search for signs of the same gravity as in the severe type, and the use of insensitive methods.

So far no adequate treatment is available. Fresh plasma has no effect. We have tried infusion of platelet suspensions, but without success. No platelets capable of aggregating were observed in the circulation immediately after the infusion. Other authors have reported a slight transitory effect [7, 12]. Our patient might have been sensitized to homologous platelets by previous blood transfusions. If so, it would explain the poor result.

In thrombasthenia the bleeding symptoms appear to be less severe than those in essential thrombocytopenia, probably because the platelet factor 3 in thrombasthenia is normal and the clot that forms is therefore better. Careful haemostasis is thus important, and it is possible that E-ACA or cortisone may have a certain nonspecific favourable effect. To minimize menstrual bleedings treatment with ovulation inhibiting hormones may be tried.

## Summary

Three cases of severe thrombasthenia in a Swedish family are described. Two were sisters, the third was a female cousin. The most impressive abnormality *in vitro* was a complete absence of the ability of the platelets to aggregate and to adhere to glass. It was not possible to normalize the defect by infusion of platelet concentrate, fraction I–0 or fresh exercise-activated plasma. Cortisone shortened the bleeding time but had no effect on the adhesiveness. E-ACA appeared to have a favourable effect on the bleeding tendency in connection with tooth extraction.

The ancestors could be traced back through 6 to 9 generations. Thirty relatives were investigated and in nine of them moderate bleeding symptoms, prolonged Ivy bleeding time and usually a moderately decreased platelet adhesiveness were found. They were probably carriers of the gene for severe thrombasthenia in a single dose. The term mild thrombasthenia is suggested for this condition.

## Acknowledgement

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# Extinction or Explosion of the Population

## *Birth Control in Sweden with Particular Reference to its Consequences and Significance for Population Development*

*by* CURT GYLLENSWÄRD

Most of the countries of the world are threatened by a population explosion. Birth rates are high, and at the same time infant mortality figures are declining The most important factor to have exercised a regulatory effect hitherto on the growth of population has thereby been weakened. More and more mouths have to be fed, more and more people are demanding a share of a cake that is already not big enough to go round. The process of development seems to be involved in a vicious circle—all the more tragically since it originated in a naturally desirable improvement in the conditions of life for human beings. There are many who believe that it is impossible to influence this development at any rate within the time that we can reasonably assume we have at our disposal, and that a catastrophe is inevitable For various reasons it is believed that birth control is difficult or impossible to carry out in these countries, and that even if it could be practised it would result in the extinction of the nations concerned. However our experience in Sweden seems to show that there is no justification for such a pessimistic view This experience may therefore be a matter of worldwide importance Sweden —together with Finland —can offer unique statistical material, data on population statistics having been accessible ever since the year 1750

### *General development of population in Sweden*

Within the present frontiers of Sweden, average population during the latter half of the 18th century in round figures, was 2 million. On an average about 0 000 living children were born to this population every year Of these children however 14,000 (*0 ) died during their first year of life.

A hundred years later the population had doubled. There were twice as many Swedes, and they had 130,000 children born alive Only 13 (16 000) of these children died in infancy (Table I)

If the birth rate had been as high at the end of the 19th century as it was at the end of the 18th, at least 10,000 more children would have been born alive every year But if infant mortality also had been as high 26,000—instead of 16,000—of the babies born would have died in infancy and the two figures would have cancelled each other out

TABLE 1 *Development of population in Sweden 1751–1960*

| Year | Average population | Live births | Deaths in 1st year of life | Deaths at all ages | Surplus of births | Population increase |
|---|---|---|---|---|---|---|
| 1751 1800 | 2,085,700 | 69,319 | 14,106 | 56,501 | 12,818 | 11,333 |
| 1801 1850 | 2,790,035 | 89,922 | 15,473 | 66,685 | 23,237 | 22,703 |
| 1851 1900 | 4,342,735 | 130,190 | 16,344 | 80,246 | 49,944 | 33,078 |
| 1901 1950 | 6,001,300 | 116,644 | 6,815 | 75,271 | 41,373 | 38,196 |
| 1951 1960 | 7,285,643 | 107,050 | 1,938 | 70,567 | 36,483 | 45,614 |

Some reduction in the birth rate—by 7%—had occurred, but there had been a relatively fivefold greater fall in infant mortality. A population explosion was on the way. But heavy emigration, equivalent to 40% of the surplus population, reduced this.

In the first half of the 1900s the average population increased to 6 million, while the number of live births declined to 117,000. If the birth rate had been as high as it was in the second half of the 18th century 210 000 living children would have been born annually; if it had been as high as it was at the end of the 19th, 195,000. Of these 117,000 however barely 7000 died as babies. Immigration had now begun, still with an insignificantly larger number of emigrants than immigrants, but the final result—mainly as a result of the continued steep decline in infant mortality—was a larger natural increase in population than ever before despite substantial birth control.

In the ten year period from 1951 to 1960 the average population rose to 7¼ million, with 107,000 live births, while only 1900 babies died in their first year of life. At the same time there were more immigrants than emigrants. In 1965 the national infant mortality average was a mere 1.4% as compared with over 20% two centuries earlier.

Not only infant mortality has fallen. General mortality has declined also and consequently the average lifetime has lengthened. At present it is not quite 72 years for men, and almost 75 years for women.

The final result of all these changes, however has been that despite such widespread family limitation that for example during the ten year period 1951–1960 only 107 000 children were born annually instead of the 241,000 that would have been born if the birth rate had been as high as it was at the end of the 18th century the population has increased at approximately the same rate for 900 years. Emigration and immigration have been factors of major importance only for brief periods, and even then the part they have played in the whole picture has been a subordinate one. Two factors have been dominant: on the one hand birth control, on the other the reduction in infant mortality. The former is feared as leading to the downfall of the nation, the latter as the beginning of a population explosion. Neither of these fears has been realized. In reality the two factors, generally speaking, have balanced each other. A closer study of the situation would therefore be of very great interest.

*When did deliberate birth control start and who began to practise it?*

We have already shown that demonstrable family limitation—birth control—was practised in Sweden at least as early as in the second half of the 1800s. All experience shows that phenomena of this

TABLE 2 *Changes in the relative frequency of births in Sweden, 1891–1930 in percentages of the birth frequency in 1881–1890*

| Years of age | Married 1891–1900 | Married 1901–1910 | Married 1911–1920 | Married 1921–1930 | Unmarried 1891–1900 | Unmarried 1901–1910 | Unmarried 1911–1920 | Unmarried 1921–1930 |
|---|---|---|---|---|---|---|---|---|
| 15–20 | +10 | +22 | +17 | +18 | +42 | +110 | +146 | +147 |
| 20–25 | +3 | +1 | +11 | +26 | +11 | +47 | +31 | 6 |
| 25–30 | −2 | −6 | −23 | −41 | −5 | −4 | −15 | −41 |
| 30–35 | −6 | −14 | −33 | −51 | −9 | 14 | −28 | −54 |
| 35–40 | 7 | −17 | −35 | −56 | 8 | 17 | −30 | −57 |
| 40–45 | −9 | −23 | −40 | −61 | −9 | −19 | −33 | −58 |
| 45–50 | 18 | −34 | −52 | 68 | −14 | 29 | −37 | −63 |

kind begin in the more highly educated social groups and then work their way downwards.

The age of the women concerned is therefore of greater importance than membership of a particular social group if we are to consider the question of who first began to practise birth control.

It is evident from the above that the decades from the end of the 1800s until the early 1900s are of the greatest interest if we are to seek the beginnings of birth control and to follow the course of its development. There is a widespread belief that family limitation first began to be practised by young women. This idea however is a false one. Statistical data show incontrovertibly, on the contrary, that it was women of the highest ages at which it was possible for them to have children at all who first began to practise birth control (Table 2). It then spread to younger women and at the same time became more widespread among those age groups in which it had already been practised previously.

The figures for the higher ages were so high even in the 1800s that it can be asserted without risk of error that birth control had been practised decades earlier on a considerable scale at those ages. Married and unmarried show the same behaviour patterns. The tendency is so obvious and far reaching that there can be no doubt that it is a question of voluntary family limitation. This becomes still more evident if we reverse our inquiry and examine not those who practise birth control, but those who bear children. This is of decisive importance also in considering the question of whether birth control leads to the extinction of a nation.

It is a fact of particular importance that making propaganda for contraceptives or giving instruction in technics of contraception was legally almost prohibited in Sweden until 1926 and until 1938 only allowed with strict limitation—not even medical students were so instructed. Nevertheless, contraception was practised, and to a considerable extent. If a law and/or a religious taboo is regarded as unjustified, out of date, or in conflict with the reasonable demands of women for an existence worthy of self-respecting human beings, it is not respected but is evaded in one way or another.

Nearly 60% of all the children of married women are born to mothers aged 20 to 30 years, and a further 25% of such children

TABLE 3 *Percentile distribution of puerperal women in Sweden by ages*

Live births.

| | Legitimate (ages) | | | | | | | Illegitimate (ages) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15–19 | 20–24 | 25–29 | 30–34 | 35–39 | 40–44 | 45–49 | 15–19 | 20–24 | 25–29 | 30–34 | 35–39 | 40–44 | 45–49 |
| *Year 1921–30* | | | | | | | | | | | | | | |
| Rural areas | 1.7 | 18.0 | 27.6 | 24.7 | 18.2 | 9.8 | | 22.5 | 42.6 | 18.3 | 8.6 | 5.2 | 2.4 | |
| Towns | 1.6 | 19.3 | 30.6 | 23.[illegible] | 16.1 | 7.0 | | 16.0 | 44.6 | 20.9 | 9.2 | 5.0 | 2.1 | |
| *Year 1946–47* | | | | | | | | | | | | | | |
| Whole country | 4.5 | 23.9 | 31.0 | 22.3 | 12.1 | 3.6 | 0.3 | 39.0 | 34.4 | 12.4 | [illegible] | 4.2 | 1.6 | 0.1 |

are born before their mothers are 35 years old (Table 3). Marital age, which has fallen somewhat in Sweden during recent decades, is today in Sweden about 23 years for women and 26 for men in the case of the first marriage. It is thus the 10 years thereafter that are of importance from the point of view of the birth rate.

We are now concerned with the lowest ages to which the practice of birth control has penetrated, or expressed in other terms, with the question of whether women have to an increased extent stopped wishing to have children at all.

If we investigate the number of child bearing women among married women in general, we find that there has been no decrease in the ages concerned since 1935 (Table 4). And it has of course just been shown that it is precisely the desire to have children at those ages which is important. Older women had already ceased to wish to have children long before 1935. This table too shows clearly that it is a case of a voluntary birth control. It is quite unrealistic to assume the existence of an increased involuntary sterility which would not have affected also the women between 18 and 35 years of age.

TABLE 4 *Ratio of puerperal women to 100 married women in Sweden*

| Age groups (years) | 1920 | 1925 | 1930 | 1935 | 1940 |
|---|---|---|---|---|---|
| 15–19 | 64.6 | 60.3 | 47.6 | 51.2 | 60.4 |
| 20–24 | 42.0 | 26.4 | 30.7 | 27.[illegible] | 28.9 |
| 25–29 | 29.6 | 16.3 | 20.3 | 17.1 | 16.8 |
| 30–34 | 22.2 | 11.4 | 14.5 | 10.2 | 9.4 |
| 35–39 | 16.1 | 7.2 | 8.9 | 6.2 | 4.5 |
| 40–44 | 8.2 | 3.1 | 3.2 | 1.7 | 1.0 |

A very interesting circumstance referring to the great extent to which family limitation has taken place is the distribution of births according to the seasons of the year (Fig. 1). This shows a minimum in February closely followed by a 30 % increase in March, continued high level throughout the spring, a fall during the summer, a new but minor rise in September and then again a slight increase in December followed by a fall in January and the lowest level in February. This sequence recurs year after year and is similar all over Sweden. The spring peak is caused by fecundation during the summer, the September peak by impregnation at around the Christmas holidays. Despite voluntary birth control there is a factor which makes its presence felt with all the power of an elemental force of nature.

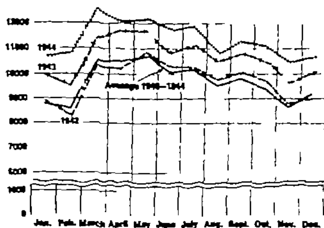



Fig. 1 Number of children born in Sweden 1940–1944.

*Favourable and unfavourable consequences of birth control and of the decline in mortality*

An obvious consequence of the fact that it is the older women who are responsible for family limitation is that the average age of mothers has fallen. Biologically this is a gratifying development.

A relatively easily measurable and very evident factor is the proportion of stillbirths. If this is set in relation t the mother age the proportion of stillbirths rises with age (Table 5) Unmarried mothers, relati ely speaking run a 50 % greater risk of giving birth to stillborn children than married mothers of the same age An increase in the age of the mother by 10 years increases the risk f stillborn children just as much, and in both groups, which shows that age is the decisive factor

A consequence of the decline in morta lity generally is that the relation between the sexes has changed for the better Of living children, 5 % to 6 % more boys are born than girls, but the boys are subject to higher mortality than the girls, and the result eventually is a surplus of females. The weaker sex, the male, has derived more benefit from the causes which have reduced mortality Propor

TABLE 5 *Stillbirths legitimate and illegitimate in percentages by mother s age 1934–193[illegible] in Sweden*

| Born | Mother age in years | | | | | | |
|---|---|---|---|---|---|---|---|
| | 15–20 | 20–25 | 25–30 | 30–35 | 35–40 | 40–45 | 45–50 |
| Legitimate (% stillbirths) | 1.5 | 1 7 | [illegible] | 2.0 | 2.7 | 4.8 | 6.5 |
| Illegitimat (% stillbirths) | [illegible] | 2.7 | 3.4 | 4.2 | 5.6 | 6 4 | — |
| Stillbirths, age 15–20 years = 100 | leg 100 | 113 | 147 | 200 | 247 | 320 | — |
| | illeg. 100 | 117 | 148 | 183 | 220 | 278 | — |
| Illeg.: leg leg = 100 | 153 | 159 | 155 | 140 | 149 | 133 | |

TABLE 6 *Total deaths per 1000 of average population in Sweden at specified ages*

| Years | 1st year of life | 15–19 years | 50–54 years | 65–69 years | 80– years |
|---|---|---|---|---|---|
| 1751–60 | 205 | 6.4 | 21.7 | 50.0 | 208.2 |
| 1801–10 | 199 | 7.5 | 26.5 | 63.9 | 262.5 |
| 1851–60 | 148 | 5.5 | 20.2 | 55.6 | 211.1 |
| 1901–10 | 85 | 4.6 | 11.4 | 32.1 | 175.4 |
| 1941–50 | 27 | 1.4 | 7.2 | 28.5 | 170.9 |
| 1951–60 | 18 | 0.7 | 5.6 | 25.9 | 163.4 |

tionally, therefore, they survive at earlier ages to a greater extent than formerly. The result has been that the age at which a surplus of women appears has risen more and more. In 1920 as many men as women were still alive in their twenties. A surplus of women thus appeared among the ages above this group. In 1940 the threshold had moved almost to the age of 40 years, in 1960 to nearly 60 years, and in 1980 we may expect no surplus of women except among those nearly 70 years old. What this means for the formation of families, for example, is obvious.

But the increase in the average length of life is not an unmixed blessing. The community has more and more older people to attend to and there is consequently a relatively greater need of facilities for care of them.

TABLE 7 *Sweden. Men and women, remaining average lifetime in years*

| Years | Birth | 15 years | 50 years | 65 years | 80 years |
|---|---|---|---|---|---|
| 1751–90 | 35 | 42 | 19 | 10 | 5 |
| 1791–1815 | 37 | 4 | 18 | 10 | 4 |
| 1831–55 | 43 | 44 | 19 | 10 | 4 |
| 1901–10 | 56 | 51 | 24 | 12 | 5 |
| 1941–50 | 69 | 57 | 26 | 14 | 6 |
| 1956–60 | 73 | 60 | 27 | 14 | 6 |

There is a widespread belief that the ever-increasing rise in the length of the average lifetime means that the people of our generation may live to the age of 100 years or more.

The Swedish population statistics show clearly what the facts are in this respect.

In two hundred years—1750–1950—infant mortality fell to one-seventh, teen-ager mortality to one-quarter, mortality among fifty year-olds to one-third, among sixty five year-olds to rather more than half and among octogenarians hardly at all (Table 6). Infant mortality today, as we have mentioned, is merely 14 ‰—only one-fifteenth of what it was at the end of the 18th century.

Expressed in other terms, a newborn child has had his average expectation of life more than doubled in 200 years, while a fifteen year-old has increased by 15 years, a fifty year-old by 7 years, and a sixty five-year-old by 4 years but an eighty year-old has just the same prospects of living another five years as he had at the end of the 1700s (Table 7). We may expect to have relatively more and more people of advanced ages, but not relatively more of them over 85 years of age. This seems to be the optimum age. And if no change in this respect has taken place in 200 years, it seems unlikely that within the foreseeable future any major changes may be feared or hoped for, however we may choose to look at the matter.

The gain that has been achieved during recent decades and which has brought with it a further increase in the average length of a lifetime has been exclusively to the advantage of people up to the age of 50.

*Is birth control old-fashioned?*

Obstetrical literature often assumes that every married couple wants to have at

least one child even if birth control is generally practised on an extremely large scale. The number of childless marriages has undoubtedly increased. If the argument were correct, involuntary sterility would thus have increased. The higher *standard of living and the depopulation of the countryside in favour of the towns* have been advanced as reasons for such an increase. As far as the standard of living is concerned, it is known that the relative number of stillborn and prematurely born children is higher—for the latter in Stockholm in the 1930s twice as high among lower socioeconomic groups than among the more prosperous—the lower the standard of living [2]. The approximately 5 of prematurely born children in Sweden account for more than 50 % of infant mortality as a whole. A rise in the standard of living should therefore, we may reasonably assume, mean a reduction in involuntary sterility also, and thus have an effect that would be the diametrical opposite of what has been assumed. An investigation into the number of entirely childless marriages in Sweden in the 1930s showed that these, relatively considered, increased twice as fast in the rural areas as in the towns [2]. Thus migration to the cities has had no sterilizing effect. The figures stated in the common obstetrical manuals regarding involuntary sterility seem to be too high. Figures of 10 %, 15 %, 20 and more are quoted. An investigation based on Finland-Swedish statistics a few decades ago showed for marriages which had been in existence for at least 15 years and in which the mother had been not more than 30 years old at the time of her marriage, a figure of merely about 3 % of entirely childless marriages [4].

Moreover it has, as mentioned above, been shown that no reduction in the relative production of children has occurred since 1935 among married women aged between 20 and 35 years.

TABLE 8. *Sweden. Distribution of population by age groups*

| Year | Below 15 years | 15–64 years | Over 65 years |
|---|---|---|---|
| 1900 | 33 | 59 | 8 |
| 1930 | 25 | 66 | 9 |
| 1940 | 1 | 70 | 9 |
| 1950 | 4 | 66 | 10 |
| 1960 | 22 | 66 | 12 |
| 19 0 | 20 | 66 | 14 |

*Fluctuations in the birth rate and their effect on the age distribution of the population*

During the present century the birth rate has varied between about 26 % and 14 % of the population. This of course is in itself an unsatisfactory method of stating the number of births, if only for the reason that no women except those of reproductive age can give birth to children, and this age is less than half of a woman's average lifetime in Sweden today. This group forms one-quarter of the total population. Furthermore the distribution of ages in the population has greatly changed in consequence of (*a*) changes in the proportion of live births, which as mentioned varies between 26 ‰ and less than 14 % of the average population, or in absolute figures more than 140 000 and less than 85,000 in different years and (*b*) the increase in the average length of life (Fig. 3).

In 1900 children under 15 years of age formed 33 of the population, old people over 65 years of age 8 %.

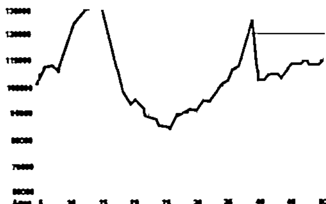



Fig. 2. The diagram shows the population structure in Sweden today

Today 20 % are children and 14 % old people. Together these two groups have comprised approximately 34 % in the last 40 years (Table 8).

But these average figures conceal a highly unfavourable distribution of ages (Fig. 2). A large group of 10- to 15-year-old is clamouring for education and is nearing marriageable age, and a large group of 35- to 40-year-olds will in a few decades be approaching retirement age and will be needing more care.

*Illegitimate children*

In the middle of the 19th century almost every other child born in Stockholm was illegitimate, in other cities a good 30 % and in the rural areas 5 %. A change gradually came about and 10 % to 12 % of children are now born out of wedlock in the whole of Sweden, regardless of whether their mothers live in Stockholm, in other thickly populated district or in rural areas. The most important factor moreover is not where the children are born but where they are conceived. Anonymity is greater in the big cities and unmarried pregnant women have always been drawn to them.

A sad fact is that the prospects of life for such children are in many respects much worse than for children born in wedlock (Table 9). Infant mortality is still relatively almost 70 % higher and no relative improvement in the proportions has occurred despite the fact that the absolute figures for infant mortality have fallen for both groups. One reason is that the frequency of premature births is higher. As the frequency of premature births is greater among women with a lower standard of living, this must prove that conditions during pregnancy are still very unsatisfactory for unmarried expectant mothers.

The essential condition for the expectant mother and her child, however, is not whether she is married in the administrative sense but whether the father and mother live together as if they were married. Two-third of the children born out of wedlock in Sweden become legitimate within two years, most of them within one year after the child's birth as a result of the mother's marriage in half of the cases to the child's father. In many countries such children are not registered

as illegitimate. Comparative figures are therefore liable to be misleading

From the point of view of social pediatrics, such legitimatized children are not illegitimate The high figure of infant mortality must naturally primarily refer to the non legitimatized children, and thus becomes still more shockingly high This is an undeniable factor which cannot be left out of account in the discussion concerning the future of the family and of marriage as institutions, their to be or not to be

*Immigration to heavily populated areas*

It has already been shown previously that immigration to densely populated areas has not connoted any increase in involuntary childlessness or greater voluntary childlessness than in rural areas

As far as infant mortality is concerned the situation is much the same In the 1940s and 1950s it was lower in the towns (Table 10) Equalization seems to have been reached only in the 1960s.

TABLE 9 *Infant mortality in Sweden among legitimate and illegitimate live births 1952–1962*

| Of 1000 live births | Legitimat | Illegitimat | Total |
|---|---|---|---|
| 1952 | 18.44 | 30.6* | 20.03 |
| 53 | 18.00 | 25.86 | 18.74 |
| 54 | 17.88 | 26.84 | 18.71 |
| 55 | 16.23 | 26.20 | 17.44 |
| 56 | 16.57 | 25.70 | 17.33 |
| 57 | 16.77 | [illegible]8.84 | 17.77 |
| 58 | 14.96 | 4.26 | 16.00 |
| 59 | 15.62 | 25.30 | 16.63 |
| 60 | 15.60 | 23.40 | 16.63 |
| 61 | 14.66 | 24.07 | 16.70 |
| 62 | 14.30 | [illegible]4.00 | 15.34 |

TABLE 10 *Deaths in 1st year of life among 100 live births*

| Years | Sweden | Rural areas | Towns |
|---|---|---|---|
| 1941–45 | 3.1 | 3.3 | 2.8 |
| 1946–50 | 2.4 | 2.6 | 2.[illegible] |

*Can infant mortality be further reduced and should it be?*

An important question is whether it is justifiable to bring infant mortality down to a figure that is as low as it can possibly be Will not sooner or later a threshold be reached beyond which children are saved only to spend their lives handicapped by severe defects?

In the situation now existing it would appear that there is still a safety margin in reserve before such a state of affairs is likely to come about Uthem Toverud's table showing infant mortality in royal families is of great interest in this connection [5]. In the 17th century every fourth child born alive in royal families still died before its first birthday but even during the first three decades of the 20th century an average of only 8 % died (Table 11) In the year 1958 infant mortality in Sweden was still almost twice as high as this for the whole country

At the beginning of the 20th century the national average for infant mortality in Sweden was about 9 %. The figures for royal families provide confirmation of a well-known fact namely that it was far higher among lower socioeconomic groups than among those who were more prosperous. In the lower socioeconomic groups in Stockholm and its surroundings the figures for infant mortality could reach and exceed 50 % in large families. But high infant mortality was paralleled by high mortality in the years of infancy and

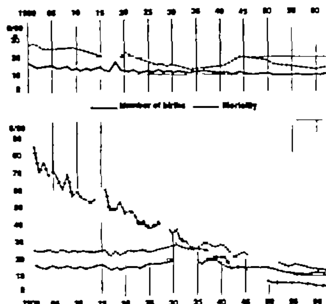



Fig. 3. Number of birth and infant mortality in Sweden 1900–1962. +—+—+— Infant mortality after first week of life Stillborn. —·—·—— Mortality first week of life.

later during childhood and adolescence. The numbers of those who were disabled for life or handicapped in their development was greater than the number of those that died. Birth control means healthier children but healthier mothers too, better able to bring up their children and with more time to devote to them. Conversely there are fewer healthier children to rejoice the hearts of their parents, and thus may have helped as we have shown, to explain the fact that general willingness to have children has not fallen off at the socially most desir able ages.

TABLE 11 *Infant mortality n royal families*
(After Utheim Toverud.)

| Years | Number of b. births | Deaths among 1000 l. births: 1st month | 2nd 12th months | 1st year |
|---|---|---|---|---|
| 1500–99 | 1852 | 93 | 104 | 207 |
| 1600–99 | 2028 | 96 | 150 | 246 |
| 1700–99 | 1907 | 60 | 93 | 153 |
| 1800–49 | 748 | 50 | 46 | 96 |
| 1850–99 | 611 | 17 | 23 | 40 |
| 1900–30 | 573 | 5 | 3 | 8 |

*Where is the margin for a further reduction in infa t mortality?*

The biggest improvement in the reduction of mortality among infants has been made during the latter 51 weeks of the first year of life (Fig 3)

Mortality in the entire year has fallen during the 20th century from—in round figures—9 % t the turn of the century to 1 4 in 1965 or by five-sixths. Th proportion of children born dead and of those dying during th first week of life on the other hand, has f llen much less: the percentage f stillbirths from about 2.5 to 1 5 and that of death in the first week of lif (neonatal mortality)

from 1 6 % to 1.1 % or in both cases by about one-third, though with a recession in the years 1926–1940 Three-quarters of all infant mortality occurs during the first week of life and, as has been noticed previously prematurely born children predominate heavily among the children who die.

The frequency of premature birth and its causes are therefore matters of major importance in the question of a further reduction in the number of children dying in their infancy The frequency of premature birth is higher the lower the socioeconomic status of the mothers. Likewise, illegitimate birth plays an important part in this connection.

Efforts should now be concentrated in Sweden on improved precautionary maternity care in its widest sense particularly for unmarried expectant mothers, and on prophylactic treatment for their children.

*The essential elements of assistance for mothers and children*

*Simplified uniform feeding* The appallingly high infant mortality figures probably explain in some part why when mothers for any reason could not suckle their children or had an insufficient supply of milk, it was generally the practice until twenty or thirty years ago—and even later—to prescribe an extremely complicated diet in the belief that this would combat infant mortality [6, 7 8 10]. The frequency of so many deaths experienced so close at hand, and the observation that breast fed babies came off much better were the motives for heavy propaganda on behalf of breast feeding When the teaching by object lessons was abandoned, there was a strong tendency for it to be replaced by an underestimation of the value of nursing However it is really difficult to understand why such a complicated method of feeding necessitating changes in the composition of cow's milk mixtures up to 30 times [6] during the first year of life in practice impossible to follow for the majority of mothers, was considered necessary The composition of breast milk does not change during the whole breastfeeding time after the colostral period more than it can vary during a single feeding from start to finish, or as between one mother and another

The ideal for bottle feeding must be the simplest possible prescriptions, easy to apply especially for the lower socioeconomic groups. At the beginning of the 1920s propaganda began to be made for a simplified *uniform mixture based on the half milk principle* [3] Industrial manufacture would not have been possible without such a unified mixture [1 3]. Products of this kind have now gained a complete victory in Sweden and have conquered the greater part of the market. Definite advantages are the reliable hygienic condition and the fact that the products can be modified and kept up to date in accordance with the progress of scientific research that they can be enriched with vitamins and trace elements, that they keep, and that they are easily prepared Thus simplified form of feeding has made things much easier for mothers and given them greater security It has not contrary to what was feared led to a reduction in the frequency of breast feeding On the contrary the assurance of knowing what can be done if the supply of breast milk falls off reduces an anxiety which often actually

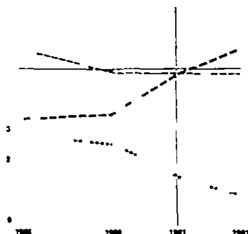


Fig. 4 Milk consumption divided into breast milk, home-cooked and factory-made formulas in Sweden 1904–1963. —— breast milk — — factory-made formulas ...... home-cooked.

contributes towards reducing a mother's capacity for breast feeding What has happened is a change from home-made mixtures to mixtures prepared from in dustrial products (Fig 4)

Simplified feeding therefore is an essential element in aid to families with children.

*Children s hospital and Child Health Cl ics throughout the country* While assist ance is given towards the care of healthy children in their homes, another funda mental principle in Sweden has been to make it possible for families to be able to rely on adequat care for their children in case of illness, no matter where they live. As there had been children hospitals only in a few cities for a long time efforts were made to set up hospitals, and thus to make children doctors available in t least one t wn in every county [5] This work began in earnest only 20 years ago but it was crowned with such success that the goal has now been achieved. These children s clinics are a model and a support for prophylactic child care also

*Obstetric clinics throughout the country* During the years 1926–1940 mortality in connection with childbirth showed a dis turbing increase both among mothers and in the number of stillbirths and neonatal deaths [2]. The less well trained the per sonnel the more unsatisfactory was the situation. The attention of the authorities was drawn to the facts their co-operation was secured and in spite of resistance from certain quarters at first obstetric care was extended to that besides children s departments under their own head physi cians with specialist training rapid prog ress was made in the setting-up of child birth departments in the provinces, also with their own specialist trained head physicians, and carrying on intimate collaboration with the child health clinics. This is the third essential element in child and maternity welfare

## Summary

The experience gained in Sweden shows that with a rising standard of living and with increasing enlightenment, women desire birth control and that they put it into practice despit obstructive legisla tion and religious taboos. This family limitation does not reduce their wish to have a limited number of children at the socially most desirable ages. It means fewer but healthier children, makes it possible for mothers to give more atten tion t their children and fatigues them less factors which in themselves ppear to promote the desire for family limitation. Lower figures for infant mortality seem

to have a similar effect and thus reduce what would otherwise be the imminent danger of a population explosion. It is the duty of the community to support mothers by providing access to obstetric care with highly trained representatives and to children's doctors and children's hospitals. Simple and unified infant feeding when breast milk is unobtainable or insufficient is an important element in this support.

Infant mortality can be further reduced even in Sweden without the risk of endangering the soundness of the national stock. The margin in reserve is considerable and covers primarily the frequency of premature births, the stillbirth rate and mortality in the first week of life.

The course of development may be traced back to the middle of the 19th century as far as birth control is concerned, but the biggest gains as regards the essential elements in assistance to mothers and children were not achieved or carried out until the last 20 years.

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CASE REPORT

# Congenital Sucrase and Isomaltase Deficiency with Temporary Lactose Intolerance

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Since the first report of sucrose and isomaltose intolerance by Weijers in 1960 [12] over 60 cases have been noted [10]. In less than a third of these cases has the diagnosis been confirmed by enzyme estimations on the small intestinal mucosa. The clinical manifestations vary from severe diarrhoea in very early infancy to milder chronic diarrhoea in older children and the condition has been recognised in a few adults.

A further example of congenital absence of sucrase and isomaltase is reported in this paper. Several additional features of this case are of interest. Lactase deficiency was also present and this was considered to be secondary to infective enteritis. The associated intolerance to lactose diminished when the infection was controlled. The diagnosis was based on dietary exclusion of the offending carbohydrates, oral loading tests and assay of enzymes on small intestinal mucosa.

## Methods

The chromatography of urinary and faecal sugars and sugar loading tests were performed as described by Arthur et al. [3].

The presence of isomaltose (which has the same $R_f$ as lactose in the solvent system employed) in stool was demonstrated by incubating an aqueous solution of the deionised acetone extract of faeces with bacterial lactase. There was no diminution of the intensity of the spot nor release of glucose or galactose on subsequent chromatography.

The disaccharidase activities were assayed as described by Arthur [2].

Blood sugars were determined by a modification of the method of Folin & Wu as described by Wilkinson [13].

## Case Report

D. S. (male) born on 28 October 1963 at term, weighing 2948 g to young healthy unrelated parents. A 20-month-old female sibling is well. The patient was breast fed for the first three weeks, stools were normal and he gained weight. Feeds were then changed to a full cream powdered milk with added cane sugar and within 48 hours he developed watery explosive diarrhoea. The stools improved when milk feeds were stopped and clear fluids were given. The diarrhoea recurred on reintroduction of milk and he was admitted to hospital. A diagnosis of infective gastro-enteritis was made. He was treated with ampicillin, streptomycin and clear fluids by mouth and again improved. No pathogens were isolated from stool cul

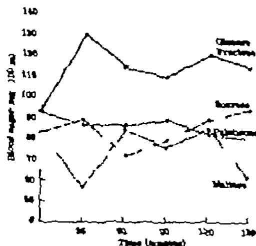



Fig. 1 Oral monosaccharide and disaccharide load tests showing rise in blood sugar after glucose and fructose and no rise with disaccharides.

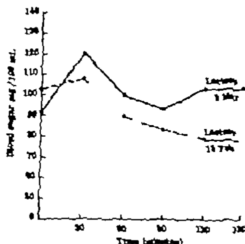



Fig. 2. Oral lactose load tests carried out imme diat ly after eradication f enteric pathogens and three weeks later showing improvement in the ability t hydrolyse the disaccharide.

tures, haemoglobin and white cell count were normal. The urine contained 30 mg/100 ml of protein and the blood urea was 120 mg/100 ml. Milk feeding again caused deterioration and he became more dehydrated with oliguria. The blood urea rose to 160 mg/100 ml and the serum sodium was 175 mEq/l.

He was transferred to The Hospital for Sick Children as a case of renal failure on 25 December 1965. On admission he weighed 2780 g and was severely dehydrated, the skin possessing a doughy consistency and he passed frequent, explosive green stools. There was no hepatosplenomegaly and the kidneys and bladder were not palpable. The external genitalia were normal. Urine was passed soon after admission with a good stream and no straining. Central nervous system examination was normal.

The dehydration and electrolyte imbalance were corrected by intravenous fluids and electrolytes over a 48 hour period and the blood urea fell to 32 mg/100 ml. No sugars were detected on urine chromatography. Stool culture grew specific *E. coli 0128* which was treated with oral colistin sulphate. Full cream powdered milk and cane sugar were recommenced but he remained ravenously hungry and he passed 10-14 watery stools daily. The stool pH did not fall below 6.5 but on chromatography varying abnormal amounts of sucrose were detected in the urine and stool. A further specimen of stool contained isomaltose and lactose in addition to sucrose; the colistin sulphate tablets given at this stage contained lactose as a base. Sucrase-isomaltase deficiency was suspected and feeds were changed to a powdered milk containing less than 0.1% lactose (Galactomin-Trufood Formula 17) with vitamin supplements. Cereals were omitted from the diet and as a result of this change there was some improvement in the diarrhoea. Further stool cultures grew specific *E. coli 0126* and *Salmonella typhimurium* which were treated with lactose free colistin sulphate. These pathogens were eradicated and the stools became less frequent and more formed. Chicken and rabbit were given in addition to milk. Progress was satisfactory and on 17 Feb. 1966 he weighed 5670 g.

Load tests with isotonic solutions of glucose (5 g) and fructose (5 g), lactose (10 g) (twice), maltose (10 g), palatinose (15 g) and sucrose (10 g) were then carried out. Following the palatinose load (a biosynthetic sugar which is hydrolysed in the same

TABLE 1 *Disaccharidase activities of upper small intestinal mucosa*

| | Units/g tissue wet weight | Normal (Arthur A.B [2]) |
|---|---|---|
| Sucrase | Nil | > 3 0 |
| Palatinase | 0.17 | > 1 0 |
| Lactase | 1.85 | > 2.5 |
| Maltase | 4.81 | > 10 0 |

as isomaltose), palatinose was found in the urine (80 mg/100 ml) H was not upset by glucose or fructose but following the other sugars diarrhoea occurred, sucrose causing the most severe upset The results of these tests are shown in Figs. 1 and 2. His feed were then changed to full cream powdered milk with added glucose which he tolerated well.

On 12 March 1966 peroral upper intestinal mucosal biopsy was carried out. The mucosa was normal microscopically The results of enzyme estimations showed total absence of sucrase and reduced levels of palatinase and maltase. Palatinase activity is normally 22 30% of isomaltase activity palatinose being hydrolysed specifically by isomaltase only There was some decrease in lactase activity (Table 1).

The stool transit time in both parents following a combined disaccharide load (50 g each of lactose and sucrose in 500 ml of water) was normal N excess of these di saccharides was found in the urine A sucrose load test (25 g) carried out on his 16-month-old sister showed a satisfactory rise in blood reducing substances of 60 mg/100 ml within 15 minutes of administration. There w no increase in stool transit time

## Discussion

This infant developed profuse diarrhoea within 48 hours f introd ction of sucrose at 3 weeks of age nd became severely dehydrated with hypernatraemia In spite of this upset he was [illegible] enously hungry Because of the preceding history of onset of symptoms with change in feeds from breast milk to a full cream powdered milk and cane sugar and the finding of sucrose in the stools, the diagnosis of sucrase-isomaltase deficiency was suspec ted. The diarrhoea was complicated by infection with specific *E col 0126* and later *Salmonella typh murium* which were treated with colistin sulphate Lactosuria and lactose in the stools was found and it was then realised that the antibiotic was prepared in a lactose base. The lactose in the stool and urine disappeared when the antibiotic was given in a sugar free solu tion. The stool pH did not reach the low levels expected, this could have been due either to intestinal hurry [1] or due to reduction of the enteric flora by the anti-biotics [11].

The parents of our patient were unrela ted and there was no history of diarrhoea in childhood or adult lif Combined sucrose/lactose load tests were carried out on them and failed to reveal any abnorma lity His 16-month-old sister showed a normal sucrose tolerance test. Th gene-tics of this defect have not been fully established. Occurrence in siblings is not infrequent and two examples of consan guinity in parents have been reported [6 7] An autosomal recessive mode of trans-mission is postulated [8].

The results of the load test in our case are in agreement with other reported cases of primary sucrase–isomaltase deficiency and sh wed a good rise in total reducing substances following the ingestion of glucose and fructose but no rise with sucrose maltose or palatinose However an unusual feature the different results of the t o lactose load test the first

being flat and the second, carried out three weeks later showing a satisfactory rise in blood reducing substances (Fig. 2). It is postulated that the sucrase–isomaltase deficiency is primary because of the complete absence of sucrase activity in the intestinal mucosa and the normal histology. The reduction in maltase activity is consistent with this diagnosis [5]; further reduction could have been secondary to the enteric infection.

The reduced lactase activity is considered to be secondary to the enteric infection but this is probably not the case with the *sucrase–isomaltase deficiency for if* the reduction of all the disaccharidases were secondary to the infection the lactase activity is usually more severely affected [10]. The presence of histologically normal mucosa would not exclude the possibility of the lactose malabsorption being a secondary phenomenon [9]. Although a second biopsy was not performed there is the evidence of the two lactose load tests that 3 weeks after the infection was controlled the patient was able to hydrolyse lactose (Fig. 2) and with no restriction of lactose in his present diet there is no diarrhoea.

## Summary

A case of congenital sucrase–isomaltase deficiency with transient lactose intolerance secondary to enteric infection is reported. The diagnosis was established by load tests and enzyme assay of the jejunal mucosa which was normal histologically. The parents are unrelated and load tests on the family were normal.

## Acknowledgements

Our thanks are due to Dr. A. Piesowicz for performing the intestinal biopsy, Dr. A. Arthur for enzyme estimations and Miss Francis for help with the dietary management.

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CASE REPORT

# Salt Losing in an Infant with Congenital Adrenal Hyperplasia and Normal Aldosterone Production

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The role of aldosterone in salt losing congenital adrenal hyperplasia (CAH) has remained controversial for a long time but there is now strong evidence that salt-loss in patients with the salt losing type of CAH (21 hydroxylation defect) can be adequately explained by a severely impaired production of aldosterone

We should like to report here a newborn infant with the "non salt-losing" variant of CAH as indicated by an apparently undisturbed production of aldosterone, who demonstrated evidence of negative sodium balance When treatment with cortisol was started sodium balance became rapidly positive

## Case Report

The baby was the second child of young and healthy parents. The first child a girl, was one year old and in normal condition. Pregnancy was normal, but for unknown reason the baby was born two months prematurely Birthweight was 400 g. length 46 cm (body surface 0.18 m ) There was marked virilization of the genitalia externa with an enlarged clitoris and a high degree of labioscrotal fusion, resulting in a slit-like meatus at the base of the phallus. Sex chromatine was positive, and chromosome cultures indicated a normal female (XX) pattern with 46 chromosomes (we acknowledge the help of Prof. Dr G J P A. Anders, Dept. Anthropogenetics, State University Groningen, The Netherlands).

Urinary 17 KS were increased: 5 1 6 and 3.8 mg/24 h at 1 5 and 10 days after birth. Urinary 17-OHS [1] were –6, 3 9 and 4 1 mg/24 h respectively Urinary pregnanetriol was increased 0.17 mg/24 h [8].

The diagnosis of congenital adrenal hyperplasia, 21 hydroxylation defect was made and the infant was started on a humanized milk formula (Almiron, Nutricia, The Netherlands) to study sodium balance (Fig 1). One day after birth serum sodium concentration was 138 mEq/l serum potassium 5.8 mEq/l. Three days after birth values were 136 and 5.4 mEq/l. Six days later serum sodium was 129 mEq/l. On a relatively low sodium intake of 3.3 mEq/24 h the infant could maintain only marginal sodium balance During this period, at the 22nd day after birth, cortisol and aldosterone secretion rates (SR) were estimated, using isotope dilution techniques as described before [7]. Cortisol-SR was 1 78 mg/24 h. aldosterone-SR 280 μg/24 h. During very low sodium intake (low sodium milk, Nutricia. The

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Netherlands) of 0.5 mEq per 24 h sodium balance became negative and serum sodium dropped to 123 mEq/l. At this moment cortisol production was 5.1 mg/24 h and aldosterone production 570 µg/24 h. These increased aldosterone secretory rates indicated that the baby was not typical salt-loser" W postulated that the negative sodium balance was due t the mineralocorticoid antagonizing activity of progesterone and 17-hydroxyprogesterone. Treatment with cortisol, orally 3 10 mg per day was started on a sodium intake of 2.3 mEq/24 h. Within 10 days serum sodium rose from 123 to 138 mEq/l. The weight gain was remarkable (37th day 2620 g 47th day 2920 g) The baby was discharged from the hospital at the age of 2 months in excellent condition, on cortisol treatment, orally 3 2.5 mg per day Weight was 3650 g. Urinary 17 KS were 0.56 and 0.60 mg/24 h.

## Comment

Blizzard et al. [3], Lieberman & Luetscher [16], Mattox et al. [17] and New et al. [18] found low to normal excretion of aldosterone in salt losing patients with CAH (21 hydroxylation defect) with no increase on salt-deprivation Bryan et al [4] using the double isotope derivative method of Kliman & Peterson [13] were the first to report extremely low values for aldosterone production in five patients with the salt-losing type, receiving adaequate sodium replacement. With sodium depletion the secretion rates increased only slightly

Degenhart et al. [7] using isotope dilution methods estimated aldosterone and cortisol secretion rates (SR) in nine infants and children with CAH (21-hydroxylation defect), three with the salt losing type Cortisol-SR were determined before and on the third day of stimulation with ACTH. Aldosterone-SR were determined before and after salt-deprivation for 4–6 days, when signs of depletion were evident All patients, except a 2 month-old girl were treated for some period with glucocorticoids prior to the investigation, steroids were withdrawn 2–5 days before the secretion rates were estimated. Cortisol SR before ACTH were in the normal range for 'non-salt-losers (5.5–34 mg/ 4 h, 13–23 mg/m²/24 h) low normal in two salt losers" (4.2 and 5.9 mg/24 h, 9 0 and 12.3 mg/m²/24 h) and extremely low in one 3-month-old "salt-loser" (<0.5 mg/24 h). ACTH raised cortisol-SR in all "non salt losers" (17–104 mg/24 h, 22–177 mg/m / 24 h) but the increase was less than could be expected unde normal conditions and the relative defect in the biosynthesis of cortisol was demonstrated in this way Cortisol-SR after ACTH in the salt losers were distinctly lower as compared with the "non-salt losers (1.9–10.8 mg/ 24 h, 7.3–16 0 mg/m²/24 h) Aldosterone-SR were normal in the non-salt-losers" (60–125 µg/24 h, 38–200 µg/m /24 h) and there was a normal increase after salt deprivation (100–380 µg/24 h, 114–427 µg/m /24 h). Extremely low values were found in the three patients with the salt losing type (<1 0 1 7 and 8.5 µg/24 h) and in two patients no increase was obser ved during salt deprivation. Normal or subnormal cortisol-SR have been reported earlier in patients with the salt losing type of CAH (21 hydroxylation defect) by Kenny et al. [12], Bertrand et al [2] and Jailer et al. [11] Within one year after our publication three groups of investiga tors, using doubl isotope derivative methods, have amply supported our findings nd reported low secretion and/or excre tion of ldosterone(-metabolites) in a num ber of patients with the salt losing type

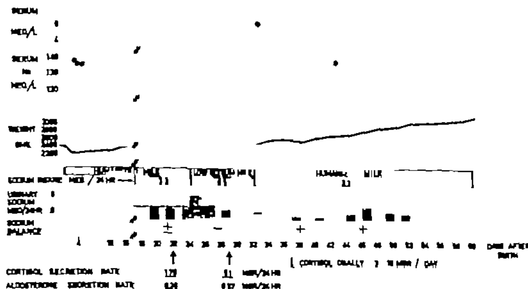



Fig. 1 Cortisol and aldosterone secretion rates during sodium balance study in infant with congenital adrenal hyperplasia (21-hydroxylation type)

of CAH (21 hydroxylation defect) [5, 14, 19]

Patients with the two variants of 21 hydroxylation defect, with and without salt loss are different genotypes [6, 20]. The results of recent studies suggest that biochemically too both types of patients represent different types of the same disorder. We have postulated that the differences in production of cortisol and aldosterone in both groups of patients, before and during stimulation with ACTH and during salt deprivation, respectively, could be explained by two different genetically controlled changes of the 21 hydroxylation process.

Assuming different changes in the 21 hydroxylation mechanism, the hydroxylation of progesterone to deoxycorticosterone (DOC) seems to be particularly defective in the salt losing type of CAH. In the variant without salt loss, the hydroxylation of 17 hydroxyprogesterone *is* apparently more defective than that of progesterone. In normal steroid biosynthesis, differences in enzyme kinetics, Michaelis constant (KM) and maximal velocity (VM) for both substrates (progesterone and 17 hydroxyprogesterone) have been described by Sharma & Dorfman [1]. One can visualize that different changes in the enzyme molecule either at the same site or at different sites, would increase those differences in enzyme kinetics and result in a greater interference of hydroxylation of one substrate [7].

17 Hydroxyprogesterone and progesterone, which are produced in increased amounts in patients with a 21 hydroxylation defect in the biosythesis of cortisol, antagonize the renal tubular effect of mineralocorticoids [10, 16], and these steroid precursors may further limit renal conservation of sodium in such patients with congenital adrenal hyperplasia.

Increased aldosterone-SR have been reported *in older patients with the* "non-salt losing" variant of CAH (21 hydroxy

lation defects) [9 14]. This may indicate that "non-salt losers" who are never treated or without treatment for a long time, produce increased amounts of aldosterone as a compensation for the mineralocorticoid antagonizing effect of progesterone and 17-hydroxyprogesterone. During the newborn period and in infancy sodium intake is usually rather low particularly when the babies are fed with human milk or humanized milk formulas. It is possible, as our case report indicates, that young infants with CAH either untreated or insufficiently treated, demonstrate symptoms of negative sodium balance while aldosterone secretion is normal or even elevated. Such babies are essentially "non-salt-losers". They will probably never demonstrate symptoms of salt loss during later life, when sodium intake is relatively much higher even when treatment has been discontinued.

It is quite possible that some of such babies are regarded as "typical salt losers" and will be treated for a long time, even years, with DOCA and salt. Estimation of aldosterone production in infants with CAH and symptoms of negative sodium balance is recommended.

We like to emphasize that infants with CAH whether "non-salt losers" (with normal aldosterone production) or "typical salt-losers" (with very low production of aldosterone) should be given adequate supplies of sodium in the diet.

## Summary

A newborn infant with congenital adrenal hyperplasia (21 hydroxylation defect) could maintain only marginal sodium balance when daily sodium intake was 3.3 mEq per 24 h (humanized milk). On low sodium milk sodium balance was negative and serum sodium concentration dropped to 123 mEq/l. Cortisol secretion rates were normal, aldosterone secretion rates increased. It is postulated that the negative sodium balance in this baby was due to overproduction of aldosterone antagonists (progesterone and 17 hydroxyprogesterone). When treatment with cortisol was started sodium balance became rapidly positive. This baby was essentially a "non salt loser". In infants with congenital adrenal hyperplasia and symptoms of negative sodium balance aldosterone production has to be estimated, to avoid longterm treatment with DOCA and salt.

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Key words Congenital adrenal hyperplasia, aldosterone secretion rate, salt losing, sodium balance studies, plasma renin concentration

## Addendum

Plasma renin concentrations were estimated in this patient on the days of the secretion rate studies, by Dr J. J. Brown and co-workers, St. Mary's Hospital, London. On day 22 plasma renin concentration was 100 units per litre on day 29 (low sodium intake) 3000 units per litre both values being highly elevated. These results suggest that the increased secretion rates of aldosterone in this patient result from regulatory stimulation by the renin-angiotensin system

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CASE REPORT

# Diabetes Insipidus

## *Observations on the Diurnal Rhythm of Urine Flow and the Treatment with Synthetic Lysine-8-Vasopressin*

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The mechanisms involved in the normal diurnal rhythm of urine flow are imperfectly understood. It has been postulated that an increase in the rate of antidiuretic hormone (ADH) secretion may be responsible for the reduction in urine flow which occurs during the night [16].

In this paper we describe observations on diurnal urine flow in a boy with diabetes insipidus who had no demonstrable evidence of ADH secretion. We also report control of the diabetes insipidus by synthetic lysine-8-vasopressin administration in the form of nasal drops.

### Case Report

The boy aged 6 years was referred to the Royal Hospital for Sick Children, Edinburgh, in September 1962 for the investigation and treatment of polydipsia and polyuria. H was the first child of healthy parents born after pregnancy during which the mother came int contact with rubella t three months gestation but did not develop any symptoms. The delivery was normal. His birth weight was 1620 g and for the first four months he breast-fed satisfactorily The mother had noticed that he was often blue and that he cried and became distressed easily He was investigated at another hospital where diagnosis of tricuspid atresia was made At the age of four months an exploratory thoracotomy was performed and as the pulmonary arterial blood flow was considered adequate surgical treatment was not undertaken.

His management became progressively more difficult H cried excessively and would often scream and become intensely cyanosed and breathless. At the age of 18 months an aortico-pulmonary anastomosis was performed. Considerable improvement resulted from the operation and this has been maintained, though he remains mildly cyanosed and his exercise t lerance is moderately impaired.

The intense thirst was first noticed when the mother started giving him fluid from cup t the age of 10 months. His distress and irritability were aggravated when drinks were not given frequently At the age of 5 years he entered school and made good progress, though the frequent visits t the toilet caused some embarrassment When first seen by us his intake of fluid averaged five litres per day and his polyuria necessitated bladder emptying every 30 t 40 minutes during waking hours.

Both parents and two younger siblings are

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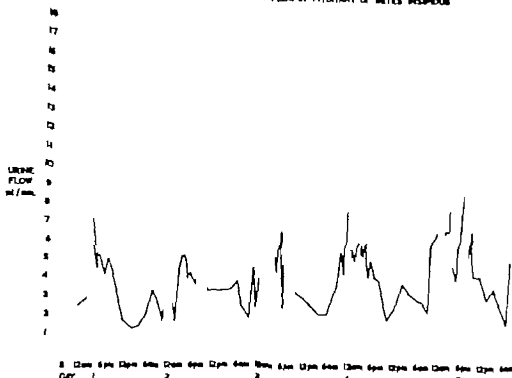



Fig. 1. Diurnal rhythm in urine flow in pituitary diabetes insipidus.

well. The maternal grandfather had polyuria and polydipsia and was diagnosed as having diabetes mellitus.

On admission his height was 104 cm and his weight 16.4 kg. He was bright and alert and of normal intelligence. There was mild generalised cyanosis with moderate finger and toe clubbing. A thrill was palpable over the sternum just below the sternal angle and a continuous machinery murmur was audible over the pulmonary area; this radiated towards the left infra-clavicular region and could also be heard over the left lung base. The pulmonary second sound was accentuated and split. There was no evidence of cardiac failure. The tip of the spleen could just be felt on palpation. His visual fields and optic fundi were normal.

*Investigation*

Chest x-ray showed mild left ventricular hypertrophy with an apparent increase in vascularity of the left lung field. On the ECG there was evidence of atrial enlargement, left ventricular hypertrophy and a partial left bundle branch block. Haematological investigation showed him to have a haemoglobin of 16.4 g per 100 ml with a P.C.V. of 47. An x-ray of the skull showed a normal pituitary fossa and no bony defects. The urine specific gravity was consistently less than 1.002. There was no proteinuria and microscopical and bacteriological examination of the urine was negative. Intravenous pyelography was performed following treatment with vasopressin and showed no evidence of structural or functional abnormality in either kidney. The B.U.N. was 16 mg per 100 ml and the creatinine clearance 125 ml/min/1.73 m. Serum potassium values ranged from 4.2 to 5.3 mEq/l. Serum inorganic phosphorus ranged from 3.9 to 4.6 mEq/l and the urinary excretion from 495–$119 Eq/24 hours. The serum calcium levels varied

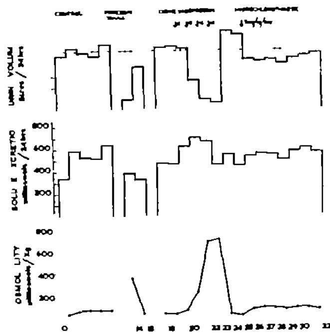


Fig. 2. Comparison of vasopressin tannat in oil, synthetic vasopressin nasal drops and hydrochlorothiazide on 24-hour urine volume solut excretion and urine osmolality

from 9 0 t 10.0 mg/100 ml. The serum sod ium levels varied from 132 t 142 mEq/l and plasma osmolality from 278 to 290 mOsm/kg. During the control phase of his investigation it was also noticed that there was a distinct rhythmicity in his diurnal and nocturnal urine flow (Fig 1).

*F nd ngs on investigation f neurohypophyseal function*

A modification of the method of Dingman *et al.* [8] was employed t assess the function of the patient neurohypophyseal system. Nicotine bitartrat was administered t test the integrity of the hypothalamic nuclei, hypertonic mannitol solution to assess osmoreceptor function, and aqueous vasopressin to determine the renal response to ADH Serial intravenous administration of each substance in turn allowed the investigation t be completed within three hours, causing minimal disturbance to the patient. The test was performed under constant water loading with intravenously administered 5 glucose and changes in free water clearance were used as an index f ADH activity The glomerular filtration rate was estimated by measurements of inulin learance.

The results presented in the table demon strate a sharp fall in free water clearance in response to a small dose f vasopressin, thus excluding nephrogenic diabetes insipidus. The administration of nicotine caused hyper pnoea, skin pallor and nausea, but care was taken t avoid more severe side effects. N reduct on in free water clearance occurred. Hypertonic mannitol raised the plasma osmolality from 275 to 290 mOsm/kg and thus also failed t result in any reduction in free water clearance The lack of evidence of ADH secretion in response t effective hypothalamic and osmoreceptor stimulation established the diagnosis of diabetes insipidus due to neurohypophyseal failure

*Treatment*

*Pitressin tannate in oil.* Following admission to hospital the boy daily urine output

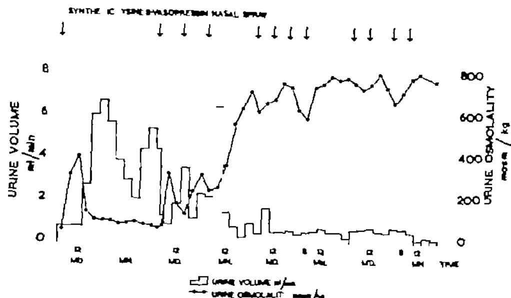



Fig. 3. The effect of a single nasal administration of synthetic lysine-8-vasopressin compared with 6-hourly and 4-hourly instillation.

on an unrestricted fluid intake ranged from 4.8 to 5.8 litres per day and the urine osmolality between 71 and 110 mOsm/kg. One unit of pitressin tannate in oil reduced his 24-hour urine output to 950 ml. The effect lasted 4 hours (Fig ) with a urine flow averaging 0 97 ml/min during the day and 0 41 ml/min at night

*Synthetic lysine-8-vasopressin.* Trial on our patient showed that each nasal instillation reduced the urine output for approximately 4 hours (Fig. 3). Six hourly administration only partially controlled the polydipsia and polyuria and only when four hourly insufflation was instituted was there a consistently low urine flow with good concentration. His daily urine output fell to 0.9 l/24 hours.

As both parents and patient were not in favour of regular injections, synthetic lysine-8-vasopressin was considered to be the treatment of choice and the boy was discharged on four hourly nasal administrations. He disliked the unpleasant sensation of the spray on nasal mucosa and nasopharynx and preferred the technique of sitting down, inverting the head between his knees and instilling two drops into each nostril. Effectiveness has varied with the care with which instructions have been followed. At times there has been mild nasal irritation and gradually a schedule was evolved in which synthetic lysine vasopressin was used when away from home and especially during school hours. The interval between administrations is lengthened after coming home when he is less inconvenienced by his polyuria and polydipsia. The last instillation is made just before going t bed Nocturia only rarely occurs now

*Hydrochlorothiazide.* In view of reports on the value of chlorothiazide and related analogues in reducing the urine volume of patients with diabetes insipidus, hydrochlorothiazide 5 mg/kg/day was given (Fig 2) [10, 13]. There was a 20 per cent reduction in the 24-hour urine volume with an initial marked increase in sodium and potassium output and a slight fall in plasma osmolality The patient did not experience any significant symptomatic relief and the hydrochlorothiazide was consequently stopped.

## Discussion

1 *Diurnal rhythm of urine flow*

Considerable speculation has centred on the reasons for the variations in water

TABLE 1 *Investigation of Neurohypophyseal Function. The ypothalamic and osmoreceptor stimulation on free water cleara*

| | Urine | | | Clear | | |
|---|---|---|---|---|---|---|
| Time min | Flow ml/min | Osmolality mOsm/kg | Plasma Osmolality mOsm/kg | $C_{H_2O}$ ml/min | ml/min | m ?3 m min |
| 0–20 | 2.6 | 88 | 282 | 1 70 | 46 | 119 |
| 20–40 | 2.5 | 98 | 277 | 1.81 | 48 | 1 4 |
| 20 | Infuse 4 m.u. vasopressin | | | | | |
| 40–60 | 1.3 | 278 | 276 | 0.01 | 42 | |
| 60–83 | 0.63 | 485 | 276 | −0.48 | 44 | 1 |
| 83–100 | 2.6 | 147 | 275 | 1 68 | 50 | 1 |
| 100–108 | Infuse 1 mg nicotine bitartrate, 0.2 mg/min | | | | | |
| 100–120 | 4.1 | 103 | 278 | 2.54 | 58 | 1 |
| 120–125 | Infuse 75 ml 15% mannitol t 15 ml/min | | | | | |
| 125–183 | Infuse 290 ml 15% mannitol at 10 ml/min | | | | | |
| 120–138 | 6.9 | 182 | 281 | 2.43 | 64 | 165 |
| 138–183 | 10.5 | 180 | 290 | 4 60 | 60 | 153 |

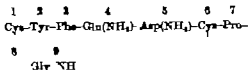
and electrolyte excretion during the day and night even when the water and solute intake is kept constant throughout the 24 hours. There is a significantly higher output of water sodium, potassium chloride and bicarbonate during the day than at night which is detectable even in the bsence of sleep [15]. That this phenomenon cannot wholly be explained by a change in ADH activity was emphasised by Goldman *et al.* [9] who measured urine volumes during periods of waking and sleeping and performed bioassays of anti diuretic substances in normal dult males as well as patients suffering from congestive cardic failure and cirrhosis of the liver Greater recovery of ADH was more often associated with the larger urine volume Our patient demonstrates that there is a distinct diurnal variation in urine flow even when there is no demon strable ADH secretion and it becomes clear that other mechanisms must play part in producing the nocturnal reduction in urine flow It does not ppear to be due to a change in 17-hydroxycorticoid or al dosterone secretion as the diurnal rhyth micity is still seen in adrenalectomised patients [7] A number of investigations have found variations in the glomerular filtration rate and renal plasma flow [17 18]. Wesson & Lauler [18] estimated that the amplitude of the variation in G.F.R was in the region of .5 per cent of the mean value over 24 hours and that it was lowest near the end of the sleeping period. It rises rapidly on waking and stays up until the beginning of sleep. Disturbance of this diurnal excretory rhythm has been found in diseases of the brain-stem and upper cervical cord even when the sleep-wake pattern and intellect are normal [7 1 ]. This has led to the conclusion that the diurnal variation is under central control independent of ADH secretion Under normal conditions, however urine flow is reduced relatively more than the G.F.R and there is a concurrent rise in urine con centration. There can be little doubt that the hormone must contribute to the reduc

tion in urine volume and that the phenomenon is thus a compound of a number of factors.

2 *Treatment*

As with other conditions requireing replacement therapy diabetes insipidus has been treated in a number of ways, none of which have proved entirely satisfactory Pitressin tannate in oil injected on alternate days had a more consistent effect than the insufflation of pituitary snuff. With the synthesis of lysine vasopressin a further therapeutic agent has become available.

The substance is one of two polypeptides showing antidiuretic activity which are found in the neurophypophysis of mammals. These polypeptides consist of a ring and side chain having amino-acid sequences identical in all positions except position 8 where the amino-acid may be either lysine or arginine.


1 2 3 4 5 6 7
Cys–Tyr–Phe–Gln($NH_2$)–Asp($NH_2$)–Cys–Pro–
8 9
Gly NH

Whereas arginine vasopressin is most commonly encountered and is the antidiuretic hormone in the human, lysine vasopressin is found only in the pig and hippopotamus. Lysine vasopressin is chemically more stable and though its antidiuretic effect is species dependent the response in man is good. Since the synthesis of the pure substance in 1957 it has become commercially available and a number of favourable reports have been made both in adults and children [1 2, 3 6 8, 14]. Side effects have been few Nasal congestion and ulceration rarely necessitate reversion to pitressin tannate in oil injections. In our patient the daily dose of synthetic lysine vasopressin varies from 30 to 40 units. This appears large when compared with one unit of pitressin tannate injected intramuscularly daily Allowance has to be made for less effective absorption from the nasal mucous membrane and considerable drug loss when this route of administration is employed. The patient naturally welcomes the freedom from injections.


## Summary

1 The investigation of a boy with pituitary diabetes insipidus is described. Synthetic lysine vasopressin applied to the nose effectively controlled urine output and thirst without any side effects.

2 Diurnal rhythmicity of urine flow is maintained even when there is no demonstrable ADH secretion. The possible mechanisms are discussed



## Acknowledgements

We wish to thank Mr E. Skedd for much technical assistance. Messrs Sandoz Ltd. kindly supplied the synthetic lysine vasopressin.

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## LETTER TO THE EDITOR

# *Glucose Tolerance in Overweight Babies and Infants of Diabetic Mothers*

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According to the recommendations of the World Health Organization 1965 a woman who has given birth to a child weighing 4.5 kg or more at birth is lassified as a potential diabetic [7]. However follow up studies demonstrate that only a certain per centage of these women later develop clinical diabetes or impaired glucose tolerance [5]. Within a group of overweight infants it would be of value to identify those who are the result of maternal diabetic influences during pregnancy The glucose homeostasis in newborn infants of diabetic mothers differs from that in infants of non-diabetic mothers [2]. There is a significant difference in the disappearance rate of intravenously admini strated glucose between these two groups of infants. This observation is the background of the present study which reports the results of intravenous glucose tolerance tests per formed in newborn overweight infants. For comparison also infants f mothers with known disturbances in carbohydrate meta bolism during pregnancy were tested.

*Material and methods.* The material con sists of 45 new born infants divided into three groups:

1 Eleven infants of insulin dependant mothers. All mothers were carefully super vised by two of us (N -O L., B P ) through out the pregnancy with the aim of maintain ing normoglycemia. Five were delivered by caesarean section and 6 vaginally The mean gestational age was 37 weeks (range 35–38) and the mean birth weight 3170 g (range 2450–3680)

2. Seven infants of mothers with gesta tional diabetes defined as an abnormal intra venous glucose tolerance ($k_G$ <1 0) during pregnancy (for methods see [5]). The mothers were selected for the tolerance test because of glycosuria during pregnancy or because of previously complicated pregnancies (large baby abnormal weight gain, antenatal death, recurrent abortion) None was given exogenous insulin. One was delivered by caesarean section, 6 vaginally The mean gestational age was 38.5 weeks (range 35–42) and the mean birth weight was 3660 g (range 3160–4650).

3. Twenty-seven infants weighing 4500 g or more at birth. All were delivered vaginally The mean gestational age was 40.3 weeks (range 39–42) and the mean birth weight was 4810 g (range 4600–5500). Birth weight ex ceeded SD for gestational age and sex in all cases [3].

All 45 infants were in good condition at the time of the tolerance test and none presented symptoms of hypoglycemia. The test were performed t an age f 1 7 days (mean 4 days) and were started 4 hours after the last feeding. 12 ml of a 20 glucose solution was given intravenously within 2 minutes. Capil lary blood samples were obtained before and every 5–10 minutes of the first hou after the injection. Glucose was determined with gl cose-oxidase [6] and the disappearance rat of glucose from the blood was calculated as earlier described and expressed as k value [4].

This work has been supported by grant from Expressens Prenatalforskningsfond, Semper Food för Näringsforskning and Karolinska Institutet.

*Results and comments* The individual results of the glucose tolerance tests expressed as $k_G$ are shown in Fig. 1 The mean $k_G$-value and standard deviations for normal infants of the same age are given for comparison [4]. As illustrated in Fig. 1 all the infants of insulin-dependant and gestational diabetics have high $k_G$-values suggesting a state of functional hyperinsulinism. In accordance with earlier observations we found that strict control of the maternal diabetes during pregnancy resulted in a reduction of the birth weights to the normal range for gestation and sex. It has been claimed that among infants of diabetic mothers there is a direct relationship between birth weight and degree of pancreatic islet hypertrophy [1]. Several theories (growth hormone, insulinantagonist, adrenal corticosteroids and maternal hyperglycemia) have been proposed to explain the pancreatic beta cell hyperplasia and increased insulin secretion capacity found in these babies (for review see [2]). If maternal hyperglycemia is the only explanation for this pancreatic hypertrophy and increased insulin secretion capacity one might expect to find a relationship between birth weight and $k_G$-value. No such correlation was found in the two groups of infants of mothers with known disturbance in carbohydrate metabolism during pregnancy There is a wide scatter in the $k_G$-values of overweight infants but 7 out of 27 have high glucose tolerances corresponding to those found in the two other groups (Fig. 1). This increased glucose tolerance might reflect an abnormal gestation of diabetic nature and thus being of predictive value

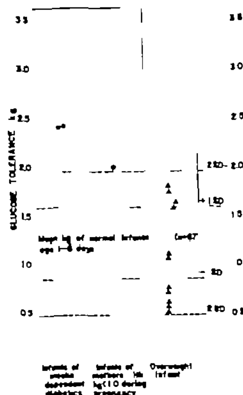



Fig 1

*J Gentz, N -O Larnell, P Ol n, B Persson, O Sterky*

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# ERRATUM

Table 1 page 640 vol. 55 1966 in the article "Fatal BCG infection in an infant with congenital, lymphocytopenic agammaglobulinemia" by L. E. Carlgren, C G Hansson, L. Henriesson and P Wåhlén should be changed as below

TABLE 1 *Results of typing tests of the mycobacterial isolates*

| Source of isolated organism | Sensitivity t | | Growth on L-J medium | | Pathogenecity guinea pig | Niacin test | Nicotin amidase activity | Trans-hydrogenase activity |
|---|---|---|---|---|---|---|---|---|
| | INH | PTH | 37 C | 22°C | | | | |
| Gastric lavage fluid | + | + | + | − | − | − | − | − |
| Spleen | + | + | + | − | − | − | − | − |
| Skin | + | + | + | − | − | − | − | − |
| Key | | | | | | | | |
| M. tuberculosis | + | − | + | − | + | + | + | + |
| | − | − | + | − | ± | + | + | + |
| M. bovis | + | + | + | − | + | − | − | + |
| | − | − | + | − | ± | − | − | + |
| BCG | + | + | + | − | − | − | − | − |
| | − | − | + | − | − | − | − | − |
| Unclassified mycobacteria | − | − | ± | ± | − | − | ± | + |

# BOOK REVIEWS

*David Yi Yung Hsia* Inborn Errors of Metabolism.

Part I. Clinical Aspects, 396 pp., ill. U.S. $11

*David Yi Yung Hsia and Tohru Inouye* Inborn Errors of Metabolism.

Part II. Laboratory Methods, 2nd edition, Year Book Publishers, Chicago 1966 244 pp ill. U.S. $7

This classical text-book f inborn errors of metabolism has now been presented in its second edition. This time it consists of two separate volumes. Part one covers the clinical aspects of the diseases and part two deals with the laboratory methods to be used in the study and diagnosis of this type of disorders. Since the first edition was published in 1959 many important developments have taken place which prompted part I to be re-written t a large extent. Some of the elementary material on biochemical genetics has also been omitted and newer information on DNA, RNA and coding has been added as well as a section on normal biochemical variations. Part two represents a completely new pproach. It is meant to meet the needs of those who are called upon to perform ppropriat tests for the detection and confirmation of the inborn errors f metabolism. The methods are described by the principle of the test how t prepare the reagents and how to perform the test The normal values and those f th diseases are also given as well as some references. In an appendix a cross index of the conditions described in part one and the laboratory methods described in part two is presented. It is of course very difficult to make a representative selection of methods as almost every field of biochemistry is represented in the book and the selection can perhaps be criticized in some details. However being the first survey of laboratory methods for the detection and study f inborn errors of metabolism part two is a very valuable con tribution and a good supplement t the first part on clinical aspects as well as to the text books f linical hemistry that are vailable Some details in a book of this type can of course alway be discussed. Thus the schematic drawing f the enzyme system which catalyzes the oxidation of phenylalanine t tyrosine seems to be wrong It is also remarkable that there is no comment on the penicillamine treatment of cystinuria, which has been very much studied and discussed during the last five years. Some typographic error also makes the last two tables in the second volume somewhat confusing How ever all these things are slight imperfections. Inborn Errors of Metabolism by Hsia in it first edition became a classical text-book of these disorders There is no discussion that it will remain so in its second edition.

*Leif Hambraeus*

## NEW BOOKS RECEIVED

*Report of a WHO Scientific Group: Clinical Aspects of Oral Gestogens.* World Health Organization: Technical Report Series, 1966, No 326. 24 pages. Price Sw F 2

*Report of a WHO Expert Committee: The Midwife in Maternity Care.* World Health Organization. Technical Report Series, 1966 No. 331. 21 pages. Price Sw F 2

*Report of a WHO Scientific Group: Basic and Clinical Aspects of Intra-uterine Devices.* World Health Organization: Technical Report Series, 1966, No 332. _5 pages. Price Sw F 2.

*Report of a WHO Scientific Group: Chemistry and Physiology of the Gametes.* World Health Organization. Technical Report Series, 1966, No 333. 23 pages. Price Sw Fr 2.

ZIEGLER, E.. *Die Ursache der Akzeleration.* Suppl. XV *Helvetica Paediatrica Acta.* Verlag Schwabe & Co., Basel/Stuttgart 1966. 94 pages, ill. Price Sw F 6.50

BECHMANN H., KUHN H. A. and MANCKE, R. *Akute und chronische Lebererkrankungen.* Georg Thieme Verlag Stuttgart 1966. 160 pages ill. Price DM 46.

BARNESS L. A. *Manual of Pediatric Physical Diagnosis.* Year Book Medical Publishers, Inc., Chicago, 1966. 238 pages, ill. Price $4.-5

HATCHER, J and JENNINGS, D. *Hypoxia. Proceedings of the Int. Symp. on the Cardiovascular and Respiratory Effects of Hypoxia.* Queen Univ Kingston, Ont., June 3–5 1965. S. Karger Basel/New York, 1966. 408 pages, ill. Price Sw Fr 96.

KUBLI, F and SPIELMANN W. *Neuere Gesichtspunkte zu Prophylaxe, Diagnose und Therapie des Morbus Haemolyticus Neonatorum.* (Bibl. Gynaecologica Fasc. 38.) S. Karger Basel/New York, 1966. 176 pages, ill. Price Sw Fr 38.

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GRAMLICH F.: *Die Rezeptorfunktion der Erythrozyten.* (Bibliotheca Haematologica Fasc. 25.) S. Karger Basel/New York, 1966. 158 pages, ill. Price Sw Fr 38.

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LEVINE, M. I and MASCIA A W. *Pulmonary Diseases and Anomalies of Infancy and Childhood.* Hoeber Medical Division, New York, 1966. 368 pages, ill. Price $12.00.

ILLINGWORTH, R. S and ILLINGWORTH, C M. *Lessons from Childhood.* E. & S. Livingstone Ltd., Edinburgh and London, 1966. 384 pages. Price 37s. 6d.

MORGENSTERN M, LOW-BEER, H and MORGENSTERN F. *Practical Training for the Severely Handicapped Child.* William Heinemann Medical Books Ltd., London, 1966. 134 pages, ill. Price 21s.

PAINE, R. S and OPPE TH. E. *Neurological Examination of Children.* William Heinemann Ltd. 1966. 279 pages, ill. Price 45s.

SPIESS, H. *Schutzimpfungen.* Georg Thieme Verlag, Stuttgart, 1966. 370 pages, ill. Price DM 45.

Acta Paediatrica Scandinavica 56: 233–242 May 1967

From the Children Department Pediatric Research Institut and Department of Obstetrics and Gynaecology Rikshospitalet, Oslo Norwa

# On Placental Transfer of Erythropoietin

by PER HAAVARDSHOLM FINNE

Previous work has shown increased erythropoietin content in blood from anemic erythroblastotic infants [1 6 7 1 29], a correlation between the degree of foetal anemia and the cord blood erythropoietin levels [7] and transfer of erythropoietin from the foetus to the amnioti fluid [6, 16]. An brupt rise in amniotic fluid erythropoietin levels is found when foetal hemoglobin drops below 11–10 g/ 100 ml although erythropoietin is also demonstrable at higher hemoglobin levels, and by concentration even in amniotic fluid from normal pregnancies [8]. A correlation between the erythropoietin content in foetal blood, foetal urine and amniotic fluid is found [6] indicating that erythropoietin demonstrated in amniotic fluid is derived from the foetus. Erythropoietin is transferred to the amniotic fluid —at least partly—through the urine [6, 16]. The lack of correlation between erythropoietin levels in foetal and maternal blood [6], supports the assumption that erythropoietin in mniotic fluid is of foetal origin and that foetal hypoxia is the stimulus for its production. This assumption is lso in greement with the work of Jacobson *et al.* [18]. They showed that the foetus in mice can initiate and maintain erythrocyte production independently from maternal erythropoietin production.

Kondi *et al.* reported that increased reticulocyte counts may be found in Rh-immunized pregnant women who give birth to severely anemic infants, and they postulated that raised reticulocyte levels are caused by erythropoietin transferred from the anemic foetus to the mother [22 23]. Similar findings have been reported also by others [10 26]. However great variations in reticulocyte counts re found in Rh-immunized women [19]. Kondi [ 4] also states that there is generally no parallelism between the maternal reticulocyte level and the severity of erythroblastosis but in certain cases increasing reticulocyt count in maternal blood in dicat an anemic foetus. Today with other suitable tests available for antenatal diagnosis of severe erythroblastosis, as spectrophotometric analysis of amniotic fluid, reticulocyt counts in the mothers are not recommended in the antenatal diagnosis of the severity of hemolytic disease of the newborn. However from a theoretical point of view it is of interest if reticulocyte levels in Rh immunized are higher than in non immunized pregnant women. A reasonable explanation of this could be that foetal erythropoietin passes

from the foetus to the mother either through the placenta or the membranes.

The present work was performed in an attempt to answer the questions: Is there any correlation between the reticulocyte counts in maternal blood, the degree of foetal anemia and the foetal erythropoetin levels in Rh immunized pregnancies? Is there higher erythropoietin levels in Rh immunized pregnant women compared to non immunized pregnant women? Is there any transfer of erythropoietin from the mother to the fortuses in mice?

## Material and Methods

Non and Rh-immunized pregnant women admitted to the Department of Obstetrics and Gynaecology for delivery were investigated. Women with pathological findings during pregnancy except Rh immunization, were excluded (pre-eclampsia, diabetes) as were women with Hb levels below 10 g/100 ml. Nearly all women had used some sort of iron medication during pregnancy

Maternal blood for erythropoietin, hemoglobin and reticulocyte determinations was obtained at the same time—between the 35th and 40th week of gestation, as in the immunized women premature induction was often performed to prevent foetal anemia from becoming too severe.

Cord blood was collected and capillary hemoglobin determinations were performed immediately after delivery

Plasma—maternal and foetal—for erythropoietin assays was frozen down and kept at −20 C until investigated.

The infants and the placentas in the normal control group were examined for normality Cases with signs of dysmaturity and placental infarctions were excluded

Ten pregnant mice at the 18th day of gestation were injected i.v with 10 U of human erythropoietin (Standard B) 3 times and with intervals of 3 hours and then killed hours after the last injection. The foetuses were taken out, washed in saline, frozen with liquid air and homogenized. Fifty ml saline was added to the homogenate and extraction performed for 24 hours at +2°C. After extraction and centrifugation the supernatant was pipetted off and the erythropoietic activity in the supernatant determined on 5 mice (10 ml) The rest of the supernatant was freeze dried after dialyzation against distilled water for 24 hours at +4°C The yield after freeze drying was dissolved in 10 ml saline and the erythropoietic activity was determined. The sediment after homogenization and extraction was injected intraperitoneally into mice which were rendered polycythemic by hypoxia for 3 weeks, and erythropoetic activity was determined Mice rendered polycythemic by hypoxia—not by transfusion—was preferred in these investigations because intraperitoneal transfusions perhaps could have interfered with the resorption of the homogenate.

To be sure that freezing with liquid air did not interfere with the erythropoietic activity of erythropoietin, the effect of the erythropoietin (Standard B) was investigated before and after freezing, and no difference in activity could be detected.

As controls served foetuses from mice at the same age of gestation. The same amount of foetal material was used for homogenization and extraction. The erythropoetic activity was determined in the same way as mentioned below

Maternal mouse plasma was collected when the mice were killed and the erythropoetic activity was determined, both in normal pregnant mice and in pregnant mice injected with erythropoietin.

The erythropoietin content was determined using polycythemic mice as recipient and $Fe^{59}$ incorporation into red cells as parameter [6]. Seventy two hours uptake of $Fe^{59}$ was used. The plasma was in some studies injected subcutaneously in doses of 0.6 ml on two consecutive days; in other studies, when doses of 1 ml 2 were given, intraperitoneal injections were preferred because subcutaneous injections of too great plasma volumes could interfere with the resorption.

TABLE 1 *Reticulocyte counts in normal and Rh-immunized pregnant women.*

Some cases giving birth to severely anemic erythroblastotic infants with highly raised cord blood erythropoietin content ($Fe^{59}$ incorporation > 9 %) were selected, and the mean maternal reticulocyte counts are shown. The difference between the control and the immunized group is statistically significant, Welch observator = 2.98, 99 % fraktil = 2.38.

| | Maternal reticulocytes, ‰ | | |
|---|---|---|---|
| | Normal | Coombs pos. infants | Coombs pos infants + increased cord blood erythropoietin levels (> 9 %) |
| Mean | 9.4 | 13.0 | 15.4 |
| Standard deviation | 2.4 | 4.9 | 6.2 |
| Number of observations | 20 | 53 | 14 |

Concentrated and unconcentrated saline extracts of mice foetuses were injected subcutaneously in doses of 1 ml on two consecutive days. Of the homogenates one intraperitoneal injection was given (2 ml).

Reticulocyte preparations were stained with brilliant cresyl blue and 5000 red cells counted in each smear [23].

A well scintillation counter was used (FH 4683 Frieseke & Hoepfner Erlangen Bruch, Germany) with a gamma sensitive crystal and with an efficiency of about 6.5 $10^4$ cp 0.1 microcurie of $Cr^{51}$ at background of 470–490 cpm.

As significance test the Welch test method was used [2]. The statistical calculations were performed by The Norwegian Computing Center.

## Results

Table 1 shows the maternal reticulocyte counts in normal controls and mothers giving birth to erythroblastotic infants with varying degrees of anemia. Some cases with highly increased cord blood erythropoietin content are selected ($Fe^{59}$ incorporation > 9 %) and the mean maternal reticulocyte counts are listed. Higher reticulocyte levels were found in the Rh immunized groups than in the controls.

In Table 2 the material is divided into different groups according to the maternal hemoglobin values and the respective reticulocyte counts are tabulated. No correlation between maternal hemoglobin and reticulocyte counts are found. The mean reticulocyte counts in the immunized group were higher throughout than in the controls, although great variations were present.

Table 3 demonstrates the results of the erythropoietin assays in maternal and cord plasma from normal pregnancies at term (Dose: 0.5 ml × 2). Significantly higher erythropoietin content was found in foetal than in maternal blood at delivery.

Table 4 shows that when higher dose of maternal plasma is injected, increased erythropoietic activity can be demonstrated. When a dose of 1 ml × 2 is used the sensitivity of the test system is great enough to demonstrate erythropoietic activity in blood from normal non-immunized pregnant women.

Table 5A shows the erythropoietin assays with plasma from women giving birth to severely anemic erythroblastotic

TABLE _. *Reticulocyte counts in normal and Rh-immunized pregnant women.*

The mothers are divided into different groups according t their hemoglobin values, and the mean maternal reticulocyte counts are shown. Numbers in parenthesis indicat number of observations. N correlation between maternal hemoglobin and reticulocyt counts is found when maternal hemoglobin is ≥ 10 g/100 ml.

| | Maternal reticulocytes, ‰ | | | | |
|---|---|---|---|---|---|
| Maternal Hb, g/100 ml | 10–11 | 11 12 | 1 13 | 13–14 | 14–15 |
| Normal pregnancies | 7 8<br>(1) | 9 7<br>(7) | 9.1<br>(6) | 9.6<br>(10) | 9 4<br>(6) |
| Rh-immunized pregnancies | 15.0<br>(9) | 11 1<br>(9) | 14.1<br>(20) | 11.9<br>(14) | [illegible]0.2<br>(2) |

TABLE 3 *Erythropoietin assays in maternal and cord plasma from normal pregnancies at term.*

Dose: 0.5 ml. 2 s.c. The difference in erythropoietin content is statistically significant, Welch observator = 6.45 99 % fraktil < _.46.

| Test material | $Fe^{59}$ uptake (% mean) | Standard deviation | Number of observations | Number of cases |
|---|---|---|---|---|
| Normal maternal plasma at term | 0.4 | 0.3 | 20 | 5 |
| Plasma from normal newborn infants | 3.2 | 2.8 | 45 | 14 |

infants in which an increased cord blood erythropoietin content was demonstrated ($Fe^{59}$ incorporation > 9 %) As controls served normal pregnant women at term. (Dose 0.5 ml × 9) Higher erythropoietin levels were found in the Rh immunized group than in the controls but the difference is not statistically significant.

Table 5B shows the erythropoietin assays in maternal plasma from normal and from Rh immunized women giving birth to erythroblastotic infants with varying degrees of anemia (Dose 1 ml × ) No difference in erythropoietin levels between the two groups could be detected.

Table 5C shows the erythropoietin assays with plasma from women giving birth to severely anemic infants in which raised cord blood erythropoietin could be detected ($Fe^{59}$ incorporation > 9 %). As controls served normal pregnant women at term. The observed difference between the two groups is not statistically significant. (Dose 1 ml × _)

Table 6 shows the results of the erythropoietin determinations in the foetuses of the erythropoietin-treated mice compared with the foetuses of the normal mice not injected with erythropoietin. The unconcentrated saline extracts in the treated group did not show higher erythropoietic activity than extracts from the control mice did, but by concentration some activity was demonstrated in extracts from both groups, and a little higher in the treated group. Higher erythropoietic ac-

TABLE 4. *Erythropoietin assays with plasma from normal pregnant women at term*

Raised erythropoietic activity is found when the dose injected is increased. The difference is statistically significant, Welch observator = 7.3, 99 % fraktil < 2.42.

| Test material | $Fe^{59}$ uptake (% mean) | Standard deviation | Number of observations | Number of cases |
|---|---|---|---|---|
| Normal maternal plasma at term Dose, 0.5 ml 2 | 0.4 | 0.3 | 20 | 5 |
| Normal maternal plasma at term Dose: 1 ml 2 | 4 | 3.7 | 40 | 11 |

TABLE 5. *Erythropoietin assays with maternal plasma from Rh immunized pregnant women*

In the groups with selected cases (severely anemic foetuses, A and C) higher maternal erythropoietin levels were found compared with the controls, but the differences are not statistically significant.

| Test material | $Fe^{59}$ uptake | Standard deviation | Number of observations | Number of cases |
|---|---|---|---|---|
| (A) The difference in erythropoietin levels between the groups is not statistically significant, Welch observator = 2.01 99 % fraktil ≈ 2.50 | | | | |
| Normal maternal plasma | Dose: 0.5 ml 2<br>0.4 | 0.3 | 20 | 5 |
| Rh-immunized women with anemic infants, cord blood erythropoietin elevated $Fe^{59}$ uptake > 9 | 0.9 | 1.1 | 20 | 5 |
| (B) The difference in erythropoietin levels between the groups is not statistically significant Welch observator 0.1.3, 99 fraktil ≈ 2.33. | | | | |
| Normal maternal plasma | Dose: 1 ml 2<br>4.7 | 3.7 | 40 | 11 |
| Rh-immunized pregnant women | 4.8 | 5.3 | 111 | 33 |
| (C) The difference in erythropoietin levels between the groups is not statistically significant, Welch observator 1.66, 99 fraktil ≈ 2.40. | | | | |
| Normal maternal plasma | Dose: 1 ml 2<br>4.7 | 3.7 | 40 | 11 |
| Rh-immunized women with anemic infants, cord blood erythropoietin elevated $Fe^{59}$ uptake 9 | 6.8 | .3 | 42 | 13 |

TABLE 6 *Erythropoietin assays with saline extract from foetuses of mice injected with human erythropoietin (Standard B) 3 times with intervals of 3 hours total 30 U in each mice.*

Erythropoietic activity in saline extract, concentrated and unconcentrated, is shown. In one group of mice rendered polycythemic by hypoxia for 3 weeks, homogenate after saline extraction was injected intraperitoneally (2 ml). As controls, served mice injected with saline (2 ml) Figures in parentheses indicate number of recipient mice.

| Test material | Erythropoietin treated mothers ($Fe^{59}$ uptak ± s.) | Normal, untreated mothers ($Fe^{59}$ uptake ± s.) |
|---|---|---|
| Maternal plasma *Dose* 0.25 ml | 35.1 ± 5.5 (6) | 3.0 ± 1.5 (5) |
| Saline extract of foetal tissue *Dose* 1 ml 2 | 0.4 ± 0.3 (7) | 0.2 ± 0.1 (7) |
| Concentrated saline extract of foetal tissue *Dose* 1 ml 2 | 1 1 ± 5.2 (5) | 7 0 ± 0 9 (4) |
| Homogenate of foetal tissue after extraction *Dose*, 2 ml i.p. | 5._ ± 1.3 (4) | 2.7 ± 0.7 (3) |
| Control, saline injected mice *Dose*, 2 ml p. | 2.3 ± 0.3 (9) | |

tivity was also found in the homogenates (after saline extraction) from foetuses of the erythropoietin treated mothers. The figures are too few for statistical calculations. However they suggest, but do not prove that small amounts of injected erythropoietin may have passed from the mother to the foetuses. Great erythropoietic activity was demonstrated in plasma from the mothers injected with erythropoietin.

## Discussion

It is well known that during pregnancy and after delivery foetal red cells may be found in the maternal circulation [3 4 10 14 21 31 34 35 36]. The immunization of the mother during Rh incompatible pregnancies also depends on this In some 30% of all pregnancies transfer of foetal erythrocytes to the maternal circulation can be demonstrated [21] though usually the amount transferred is thought to be very small. The frequency of transplacental hemorrhage vary in the different investigations [4 10, 34, 36]. However occasionally the foeto-maternal transfusion is great enough to cause foetal anemia. The high frequency of transfer of foetal cells through the placenta makes this a nearly physiologic event. During such transfusions one must assume that not only corpuscular elements, but also plasma passes. One would suppose an even easier transfer of plasma proteins than cellular elements, although based on another mechanism Many papers have been published on placental passage

of plasma proteins [5 11 12, 30] The results of these investigations, however can hardly be applied to erythropoietin, since its nature and properties are only partly known. However no close relationship between molecular size and rate of permeability is found [1 ] The molecular weight of erythropoietin, which is thought to be about 5000–50 000 cannot be said to be any objection towards its passage through the placenta [13 27 33] Foetal proteins may also pass through the membranes [32]. Theoretically a foetal influence on maternal red cell production is therefore possible when the erythropoietin production of the foetus is high due to anemia or hypoxia

The present investigations show that higher reticulocyte counts may be found in Rh-immunized than in normal pregnant women. The most likely explanation for this is that erythropoetic stimulating factors pass from foetus to mother and thus stimulate maternal red cell production. However there is great variation in reticulocyte counts both in immunized and normal non immunized women and no strict correlation with the degree of foetal anemia could be demonstrated.

In some cases with severe foetal anemia and increased cord blood erythropoietin levels, maternal reticulocyte counts were within the normal range This finding may perhaps be explained on the basis that raised foetal erythropoietin levels should be present for some time before the maternal reticulocyte level increases significantly Individual factors concerning the placental permeability may also play some role. If one assume intermittent transfer giving episodes of immunization as postulated by some investigators [*0], there could be fluctuations in maternal reticulocyte counts and a close time relationship between the foeto-maternal erythropoietin transfer and reticulocyte increase in the mother This may to some extent account for the variation found in reticulocyte counts in the maternal circulation the variations seems to be greater in the immunized than in non immunized pregnant women (Table 1) The finding of severe foetal anemia and normal maternal reticulocyte counts, makes the reticulocyte counts unsuitable in the antenatal diagnosis of the severity of erythroblastosis. Kondi [ 4] also states that there is no parallellism between the severity of erythroblastosis and maternal reticulocyte level. This is in contrast to the correlation found between foetal anemia and cord blood erythropoietin level [7]. One would expect a greater transfer of foetal erythropoietin with increasing anemia in the foetus, resulting in higher reticulocyte counts in the mother

The possibility that transferred foetal reticulocytes may interfere with the reticulocyte counts in the mother should also be mentioned. Increasing foetal anemia would tend to increase the number of transferred foetal reticulocytes, and thus increase the reticulocyte counts in maternal circulation. However increasing anemia is caused by increasing immunization of the mother and this in turn will give a shortening of the foetal cells survival time and tend to decrease the number of foetal reticulocytes in the maternal circulation [ 1 3].

Th erythropoietin assays in foet l nd maternal blood show that there are higher erythropoietin levels in foetal than in maternal blood, although maternal eryth

ropoietin levels are found to increase during pregnancy [15]. This is in agreement with the assumption that the foetus lives in an environment of reduced oxygen tension.

The erythropoietin assays in normal and Rh immunized pregnant women revealed a slight but statistically not significant difference in erythropoietic-stimulating activity in plasma from the two groups. This may perhaps indicate that the erythropoietin assay method is too crude to differentiate between slight variations in erythropoietin levels. The results of the assays, when comparing the controls with an immunized group with specially high erythropoietin levels in cord blood, could perhaps support this theory. Thus, in Table 5 higher erythropoietin content in maternal blood is found in the severely affected group (A and C). However, the difference to the normal group is not statistically significant. The maternal reticulocytes in this group also showed the same tendency: higher counts were found than in the controls (significant difference) and even higher than in the unselected immunized group (Table 1). Utilization of erythropoietin by maternal bone marrow may also interfere with the height of the erythropoietin level [9]. The dilution of foetal erythropoietin passed into the maternal circulation may make it difficult to demonstrate by direct assay. Fluctuations in the amount of transferred erythropoietin, as mentioned above, may also make it difficult to demonstrate differences in maternal erythropoietin levels.

In Table 6 are shown the results of some experimental work in mice. There are many objections which can be raised against these experiments. Human erythropoietin was used in mice, and this may interfere with the placental permeability. Results obtained in mice do not necessarily apply to human beings. Further, although the results indicate that human erythropoietin injected into pregnant mice may pass from the mother to the foetuses, they are not quite conclusive.

In conclusion one may say that higher reticulocyte levels may be found in Rh-immunized than in normal pregnant women, and although great variations, the counts are higher in pregnancies with severely anemic foetuses and with elevated cord blood erythropoietin content. The erythropoietin assays in maternal plasma did not reveal any statistically significant difference in erythropoietin content between normal and Rh immunized pregnant women. Erythropoietin levels in normal foetal plasma is significantly higher than in maternal plasma, indicating some degree of foetal hypoxia during intrauterine life, at least in the last weeks. Experiments in mice injected with human erythropoietin make it likely that some transfer of erythropoietin from mother to foetus takes place.

The reported studies on transplacental passage of erythropoietin are relatively crude. More definite conclusions could perhaps be obtained by other methods, e.g. using labelled erythropoietin. However, objections can be raised also against such technics, since the erythropoietin preparations available are not sufficiently purified.

## Summary

The reticulocyte level in peripheral blood has been determined in non- and Rh immunized pregnant women near term.

It is found higher in the Rh immunized pregnant women. This is thought to be due to transfer of erythropoietin from the anemic foetus to the mother.

Erythropoietin assays in cord and maternal plasma showed a higher erythropoietin content in foetal plasma, which is in agreement with the assumption that the foetus lives in an environment of lowered oxygen tension.

Some differences in maternal erythropoietin levels were found between non and Rh-immunized pregnant women giving birth to severely anemic erythroblastotic infants with increased cord blood erythropoietin content. However the difference was not statistically significant.

When pregnant mice were injected with human erythropoietin (Standard B) i.v. some transfer of erythropoietin to the foetuses is suggested to take place.

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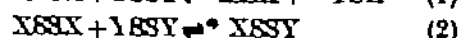
# Penicillamine Treatment of Cystinosis

by LEIF HAMBRAEUS[1] and OVE BROBERGER

Penicillamine (D-ββ'-dimethylcysteine) is a synthetic thiol compound which has a great affinity to heavy metals and has been used as a chelating agent in the treatment of Wilson's disease [16] and heavy metal poisoning [ ] It is also a sulphhydryl reducing agent and can cleave disulphide bonds. It is known that thiols and disulphides can interact and undergo exchange reactions which can be represented by the following equations



$$XSH + YSSY \rightleftharpoons YSSX + YSH \quad (1)$$



$$XSSX + YSSY \rightleftharpoons 2\,XSSY \quad (2)$$

These interactions have been studied by Kolthoff *et al.* [13] and Eldjarn & Pihl [7], and have been proposed to offer a new approach to the study of the pathogenesis and treatment of the metabolic errors of cystine [8]. Thus the effect of penicillamine treatment in cystinuria has been studied [4 5 6 9 14].

In a study of the effect of penicillamine treatment of cystinurics Eldjarn & Hambraeus [9] found that there was an increase in the urinary output of cystine. This finding was in agreement with the finding of Hartley & Walshe [1_] who made the same observation at penicillamine administration to two patients with Wilson's disease. If this situation holds true after prolonged administration of penicillamine even to patients with cystinosis, it would result in a depletion of the body stores of cystine, which might have a beneficial effect in patients with cystinosis.

In this paper the case of an infant with cystinosis is reported. Bearing in mind the possible beneficial effect of penicillamine in this disease quantitative studies of the urinary amino acids were performed during a period of penicillamine treatment and compared to the results obtained during a control period.

A preliminary report of this study has been published in 1965 [11]

## Material and Methods

The urinary specimens tested comprised 24 hr samples which were collected at the hospital using a urinary bag. No attempt was made to control the diet during the collection of the samples. The whole sample was sent to the laboratory in plastic bottles, the volume was measured and aliquots were taken and kept at −20°C until they were analysed.

The quantitative analyses of the individual amino acids were performed by means of a modified Spackman Stein & Moore automatic amino acid analyser [10, 15] The results of the chromatograms were evaluated

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according to the height times-width method described by Spackman *et al* [15].

Prior to the chromatographic analyses all urine specimens were stirred vigorously and brought to pH with hydrochloric acid. 1 ml of urine was applied to the 50 cm column and 2 ml of urine to the 150 cm column if the urine volume per day was more than 1000 ml, otherwise half the volumes were applied to the columns. A single 50 cm and 150 cm chromatogram was run on each sample of urine

The creatinine was determined by a standard method based on the Jaffe reaction, using Lloyd reagent as adsorbent and used as a control on the complete collection of a 24 hr sample

In the therapeutic trials D-penicillamine hydrochloride which was obtained from the Distillers Company (Biochemicals) Limited, Wimbledon, London S W 19 England, was used.

## Case Report

M N Boy born Oct. 20th, 1962. 3/3 siblings. Sister born 1954, healthy Brother born 1959 died at an age of 2 days from congenital heart disease Family history otherwise negative

The patient was born at full term with a birthweight of 3560 g Neonatal period uncomplicated. He was in good health up to an age of 7 months, when he started to have repeated episodes of vomiting and fever without any obvious signs of infection. He failed to thrive and weighed at an age of 17 months only 8890 g in contrast to a weight of 9000 g at the age of 11 months. Because of a history of repeated period of vomiting and fever he was suspected to suffer from urinary tract infection, but repeated urinary cultures were consistently sterile

Physical examination revealed a slightly dehydrated boy small for his age (weight 8890 g length 74 cm) Motor sensor and mental development normal. He showed signs of ricket with epiphysial enlargement of ancles and wrists, rosary and caput quadratum. The rachitic nature of the skeletal deformities was confirmed by X ray No abnormalities were noted on physical examination of heart lungs, abdomen or peripheral nerves.

On admission he demonstrated renal acidosis manifested by a blood pH of only 7 15 with a simultaneous urinary pH of 6.2. He had polyuria of 2000 ml a day The maximal concentrating capacity 17 hours following the i.m. administration of 0 15 U Pitressin tannate (Parke & Davis) was reduced to 375 mOsm/l Tubular reabsorption of phosphorous was defective with a lowering of the $Tm_p$ to a plasma level of 1 0 mg/100 ml (normal range 3.5–4 5 mg/100 ml). Further urine examination revealed glucosuria and aminoaciduria In a hydrated state the blood chemistry was normal except for hyperchloraemia of about 115 mEq/l

Because of (i) a history of repeated episodes of vomiting and fever starting at an age of 7 months in a previously healthy infant and (ii) signs of tubular dysfunction affecting both the proximal and distal parts of the tubules indicated by glucosuria, aminoaciduria, defective reabsorption of phosphorous as well as inability to acidify and concentrate the urine tentative diagnosis of cystinosis was established. Ophthalmologic examination revealed depositions of cystine in the cornea thus confirming the diagnosis.

The patient was given therapy of sodium bicarbonate 1 g ... Scholl's solution (sodium potassium citrate) 15 ml 4 and 50 000 IU of vitamin D daily On this regimen he maintained a normal electrolyte and acid base balance. His rickets showed signs of healing when controlled by x ray examination 4 months after beginning of treatment. Later however he developed a tendency towards hypercalcaemia with serum concentration up to 13 mg 100 ml. To avoid further tubular damage secondary to hypercalcaemia the vitamin D dose was lowered to 10 000 IU daily X ray examination has at no time demonstrated any sign of nephrocalcinosis

The patient did well on therapy and when re-examined in January 1966 his weight was 12 kg and his length 87 cm. However his

TABLE 1 *Amino a d excretion in the urine (mg amino acid per 4 h urs) before and dur g penicillamine adm istration*

| | Penicillamine treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 therapy | | | 1st day | | 5–7th day | | | 2nd month |
| Amino acid | | b | | | b | | b | | a |
| Taurine | 4 | — | 1 | 12 | 5 | — | 2 | 7 | 4 |
| Hydroxyproline | 22 | — | t | 39 | 14 | .3 | 40 | 28 | tr |
| Aspartic acid | 3 | — | tr | — | 1 | tr | 1 | | 14 |
| Threonine | 60 | — | 82 | 2 | 50 | — | t | 33 | 49 |
| Serine | 100 | — | 35 | tr | 44 | — | t | 35 | 43 |
| Glutamine + Asparagine | 400 | 275 | 306 | 8 | 203 | | 60 | 224 | 114 |
| Proline | 104 | 101 | 114 | — | 84 | 105 | 99 | 83 | 106 |
| Glutamic acid | 82 | 130 | 33 | 406 | 46 | 57 | 139 | 37 | 90 |
| Citrulline | 18 | 19 | 39 | 31 | 18 | 11 | | 39 | 18 |
| Glycine | 218 | 132 | 157 | 221 | 150 | 93 | 213 | 160 | 130 |
| Alanine | 187 | 118 | 147 | 202 | 176 | 127 | 161 | 131 | 118 |
| Valine | 83 | 26 | 65 | 49 | 49 | 33 | 20 | 49 | 48 |
| Cystine | 42 | — | 51 | — | 63 | — | — | 58 | 49 |
| Isoleucine | 13 | 9 | 14 | 2 | 14 | tr | — | 18 | 13 |
| Leucine | 20 | 14 | 29 | 2 | 27 | 9 | — | 24 | 27 |
| Tyrosine | 36 | — | 40 | — | 19 | — | — | 28 | 37 |
| Phenylalanine | 33 | 16 | 38 | — | 34 | 11 | 14 | 32 | 34 |
| Ornithine | 19 | tr | 18 | 41 | 10 | 3 | tr | 13 | tr |
| Lysine | 93 | 46 | 67 | 63 | 58 | 67 | 100 | 53 | 51 |
| Histidine | 85 | 56 | 60 | 25 | 57 | 36 | 41 | 85 | 63 |
| Tryptophane | 24 | — | t | — | — | — | — | — | tr |
| Arginine | — | — | 29 | — | 25 | — | — | 25 | — |

kidney function had slowly deteriorated. The daily urinary output had increased from 2000 ml in March 1964 to 3500 ml in January 1966. The maximal concentrating capacity had during the same time decreased from 375 mOsm/l to 224 mOsm/l. The glucosuria remained constant at a level of about 0.4 per cent.

During the first months in hospital an attempt was made to treat the patient with B.A.L. in order to accelerate the excretion of cystine from the tissues. This therapy however was given up, since it was found impossible to influence the plasma levels of pyruvic acid during this treatment. Instead he was given D-penicillamine hydrochloride in a dose of 30–60 mg per day in repeated periods. The amino acid pattern in the urine was studied quantitatively during this regimen.

## Results

The patient was given D-penicillamine (30 mg every 6 hours) orally during * weeks. The dose of penicillamine was then reduced to 10 mg every 6 hours, and the patient was given the drug for another * months. After a free interval of about 1 month when no medicine was administered, D-penicillamine was given once more (30 mg every 6 hours) orally during two weeks.

Table 1 shows the results obtained at the quantitative analyses of the urinary amino acids in the different urinary specimens. The specimens were collected during the control periods on 3 occasions during the

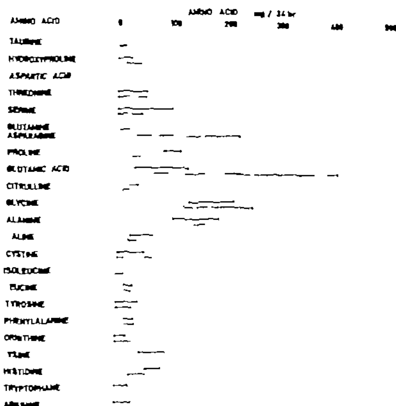



Fig. 1 Variations in the amino acid excretion in the urine before penicillamine treatment (——) and during penicillamine administration (- - )

first and fifth day after startment of penicillamine administration on [illegible] and 3 occasions respectively and after two months administration of the reduced dose of penicillamine. The values are given in mg amino acid per 24 hours It is seen from the table that there are great variations in the amino acid excretion during the control periods as well as during the penicillamine administration. It is also shown that there is no decrease in the amino acid excretion during the penicillamine treatment.

Fig 1 shows the variation in the amount excreted of the individual amino acids during penicillamine treatment and during the control periods. It is seen from the diagram that there was no significant decrease in the amount excreted of the individual amino acids nor in the variation in the excretion during the administration of penicillamine

There was no decrease in the urinary cystine excretion and there could not be found any notable amount of the mixed disulphide between penicillamine and cysteine nor of the penicillamine-disulphide in the urine during penicillamine administration to the patient

There was no positive effect to be observed in the clinical course of the disease during the administration of D-penicillamine

## Discussion

Thiol compounds such as dimercaprol and penicillamine have earlier been used in therapeutic trials in the hope of reactivating or maintaining thiol-dependant systems in patients with cystinosis [1 3] as it is known that thiol groups are essential factors in dehydrogenase systems and that there is a disturbance in the citric acid cycle in patients with cystinosis. During penicillamine administration to patients with cystinosis Clayton & Patricks [3] thus found an improvement of metabolic function nd clinical status in these patients, and the corrective influence was found to be related to the capacity of the drug to reactive impaired thiol systems.

Berger *et al* [1] published a study where they tried penicillamine and anabolic hormones in the treatment of cystinosis. Using a dose of 50 mg penicillamine per day they found a decrease in the urinary amino acid output, and the cystine excretion decreased markedly In the present study there was no significant decrease in the urinary amino acid excretion including the cystine excretion. It is also of interest that there was no increased excretion of cystine during the dministration of penicillamine and no mixed disulphide between penicillamine and cysteine or penicillamine disulphide could be detected in the urine during penicillamine treatment. These findings do not agree with the findings of Eldjarn & Hambraeus [9] who studied the excretion pattern in the urine during penicillamine administration to two cystinurics and a normal male This discrepancy might indicate that there is a disturbance in the thiol-disulphide equilibrium system in patients with cystinosis, which gives rise to an inhibition of the thiol-disulphide interactions, and hence no mixed disulphide and cysteine is formed and excreted in the urine

## Summary

A case of cystinosis in a male infant of 17 months is described.

Since part of the symptomatology of cystinosis may be a toxic effect of cystine deposits in the tissues, the therapeutic effect of penicillamine (D-$\beta\beta'$-dimethyl cysteine) was tested. This is a thiol compound which reacts with cystine converting it to a soluble mixed disulphide penicillamine-cysteine which can be excreted in the urine

It was not possible to demonstrate any definite effect of penicillamine on the amino acid excretion in the urine Nor could any measurable amount of the mixed disulphide, penicillamine-cystine be demonstrated. This latter finding might indicat that there is a disturbance in the thiol-disulphide balance in patients with cystinosis.

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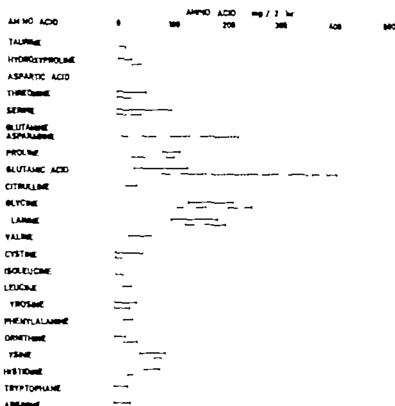



Fig 1 Variations in the amino acid excretion in the urine before penicillamine treatment (——) and during penicillamine administration (– )

first and fifth day after startment of penicillamine administration on 2 and 3 occasions respectively and after two months administration of the reduced dose of penicillamine. The values are given in mg amino acid per 24 hours. It is seen from the table that there are great variations in the amino acid excretion during the control periods as well as during the penicillamine administration. It is also shown that there is no decrease in the amino acid excretion during the penicillamine treatment

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There was no positive effect to be observed in the clinical course of the disease during the administration of D-penicillamine

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# The Normal Intrapulmonary Arterial Pattern of the Human Late Fetal and Neonatal Lung

## *A Microangiographic and Histologic Study*

by BENGT ROBERTSON

This work is the first part of a study on the pulmonary vasculature of the human fetus and infant. The present analysis of the intrapulmonary arterial pattern of the late fetal and neonatal lung will serve as a basis for subsequent comparative studies in normal early infancy and in congenital heart disease Since arterial bronchopulmonary anastomoses may serve as a shunt between the systemic and pulmonary circulations at least in conditions with reduced pulmonary arterial flow [5 14 21 26 47 48] particular attention will be paid to such anastomoses Microangiography was considered the most suitable method for this purpose since it permits subsequent histologic examination of the specimens by serial sectioning—the only reliable way of demonstrating morphologically the existence of vascular anastomoses [5 45]

The preliminary results have been reported elsewhere [40, 41].

### Previous Investigations

There is poor agreement among previous investigators concerning the incidence of arterial bronchopulmonary anastomoses in the normal human lung. Many claim to have demonstrated their existence [10 12, 13, 15 18–22 39 43 46, 49–52, 54 57], others have denied the normal occurrence of any precapillary communication between the bronchial and pulmonary arterial systems [6, 33, 44, 45]. The methods employed by these investigators were corrosion cast technique [10 15, 18, 22], angiography [2, 6, 10 22, 33, 44 55], injection with various dyes [16, 19 39 46, 57] and serial sectioning [15, 20 21 49–51 54].

Microangiographic and histologic studies on the human lung have been carried out by Lauweryns [23] and by Turner-Warwick [48]. Lauweryns reported the normal occurrence of arterial bronchopulmonary anastomoses, whereas Turner-Warwick was unable to find such anastomoses in normal lungs.

The ages of the subjects analyzed in all these studies were generally not reported.

With injection and corrosion techniques Konaschko [18] studied lungs from infants, ranging in age from birth to 1 year and reported that arterial bronchopulmonary anastomoses are usually present Tobin [47], using serial sectioning and injection techniques, observed these anastomoses, though not consistently in lungs of human subjects of varying age including newborn infants.

Arterial bronchopulmonary anastomoses were also found in the human fetal lungs and in lungs of newborn and older infants by Verloop [51] and by Weibel [54], who both used serial sectioning, and by Marchand *et al.* [29], who used the corrosion cast technique

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TABLE 1 *Distribution of birth weights in the series*

| Injection of | Birth weights, g < 500 | 501–1000 | 1001–1500 | 1501–2000 | 2001–2500 | 2501–3000 | 3001–3500 | > 3500 | Total |
|---|---|---|---|---|---|---|---|---|---|
| Pulmonary arteries | 1 | 5 | 3 | 2 | 2 | | — | 3 | 18 |
| Bronchial arteries | 1 | 4 | 4 | 2 | 1 | 2 | 4 | 5 | 23 |
| Total | 2 | 9 | 7 | 4 | 3 | 4 | 4 | 8 | 41 |

and angiography; these observations were, however, based on very small case series.

According to Wagenvoort *et al.* [52] these anastomoses occur "relatively often and close together in lungs of fetuses and infants". On the other hand, Liebow *et al.* [25] studied the human pulmonary vasculature with the corrosion cast technique and concluded that "localized congenital communications between systemic and pulmonary arteries in the absence of heart disease occur with extreme rarity". No arterial bronchopulmonary anastomoses were reported by Marini & Camarri who employed serial sectioning in their studies on the vasculature of the normal human fetal lung [30, 31].

In a recent study on human fetuses, infants and children Wagenvoort & Wagenvoort [53], using serial sectioning, were able to demonstrate occasional arterial bronchopulmonary anastomoses in some of their premature neonatal subjects.

Besides the studies by Lauweryns and Turner-Warwick on human material [23, 48], microangiographic techniques have been employed in the study of the normal pulmonary vasculature of rabbits [38], calves [37], dogs [1, 34], cats and lambs [1]. Precapillary anastomoses between bronchial and pulmonary arteries were demonstrated in the three latter species [1].

## Material and Methods

The material was selected in order to get a *fairly uniform representation of different* gestational age groups and consisted of 77 lungs obtained from 41 fetal and neonatal autopsy subjects. The birth weights ranged from 460 to 4250 g (Table 1). In one infant there was membraneous atresia of the ileum and in another there was bilobar right lung; otherwise the series includes only subjects without evidence of malformations. Fourteen infants were stillborn. The postnatal age of the others varied between 20 minutes and 7 days.

Pulmonary disorders are common in the neonatal period and often the major cause of death. Many of the subjects displayed macroscopic and microscopic evidence of pulmonary lesions such as hyaline membranes, atelectasis, aspiration, pneumonia, intraalveolar hemorrhage and edema (Table 2). These lungs cannot be considered normal but they were included in the series since they were obtained from subjects without evidence of cardiovascular malformations and without evidence of abnormal pulmonary histogenesis. Whether the diseased lungs displayed vascular changes secondary to the acute pulmonary lesions has not been analyzed in this study.

The bronchial or pulmonary arteries of the lungs were injected with 7.5 per cent aqueous suspension of fine barium sulphate ("Micropaque", Damancy & Co). The lungs were unexpanded and in atmospheric conditions during the injection procedure. In 18 subjects the injection was made into the pulmonary arteries of each lung or into the pulmonary trunc after ligation of the ductus arteriosus. The injection pressure was continuously recorded and kept around 60 mm

TABLE .. *Incidence of various pulmonary d sorders in case series (37 neonatal autopsy subjects).*

| Microscopic diagnosis | Number of cases |
|---|---|
| Purulent tracheobronchitis | 1 |
| Pneumonia | |
| Atelectasis (liveborn infants) | 5 |
| Pulmonary hyaline membranes | 7 |
| Pulmonary hyaline membranes + pneumonia | |
| Intraalveolar edema, intraalveolar and interstitial hemorrhage (liveborn infants) | 2 |
| Intraalveolar edema, intraalveolar hemorrhage (stillborn in fants) | 2 |
| Aspiration (stillborn infants) | 5 |
| Unexpanded lungs otherwise normal (stillborn infants) | 7 |
| No pulmonary lesions (liveborn infants) | 4 |
| Total | 37 |

Hg (40–80 mm Hg). In 22 subjects, the injection was made into the thoracic aorta, after ligation of the ductus arteriosus, t f ll the bronchial arteries. The injection pressure in this group was kept around 100 mm Hg (80–120 mm Hg). The injection time was generally at least 45 minutes in both groups. In few instances the injection had to be discontinued earlier because of vascular ruptures.

After injection, the lungs were fixed in 10 per cent neutral formalin for 4 to 7 days. Frontal slices of the lungs, 2 mm in thick ness, were radiographed and representative specimens were selected for paraffin em bedding Pieces were taken from all lobes of the lungs including the hilus and especially from areas with radiographic evidence of bronchial artery filling. After paraffin beeswax embedding, the selected specimens were cut in 1000–1500 $\mu$ thick blocks, which were stereo-microangiographed by a method previously described by Ljungqvist & Lagergren [*7]. An verage of 6 pairs of microangiogram measuring bout 3 4 cm were produced from each lung or pair of lungs. Areas of particular interest found in the microangiograms were cut out from the blocks and re-embedded for histologi examination. The histologic sections, 6–7 $\mu$ thick, were stained with Verhoeff lastic tissue stain. Serial sections were cut from all areas with evidence of arterial bronchopul monary anastomoses in the microangiograms and from other areas with unusual ppea rances. An average of 12 blocks were serially sectioned on each lung or pair of lungs.

Four pairs of lungs had t be excluded from the series because of incomplet filling or extensive vascular ruptures. Three of these had been injected via the aorta the fourth via the pulmonary artery In two other lung pairs injected via the aorta the bronchial arteries of only the right lung were filled. There remained, then, 67 lungs from 37 subject in which the injection was considered successful.

## Results

### *Pulmonary Arterial System*

*Basic pattern* The branches of the pulmonary artery generally follow those of th bronchi Th med llary" (circum hilar) zone of th lung however is also consistently supplied by muscular lobula arteries arising more or less perpendicu larly from the elastic pulmonary arteries (Fig 1) These abrupt branches are much narrower than their parent vessel




Fig. 1. Abrupt muscular branch from elastic pulmonary artery (PA), with recurrent pulmo-bronchial artery (PBA). Corresponding arrows in and b. Pulmonary artery injected specimen from full-term stillborn infant. Microangiogram 43. b. Selected serial section from the same area. Verhoeff 43. Diagram of the same area showing the pproximate level of b.

Fig. — Pulmonary arteriole traversing interlobular septum to ramify into alveolar capillaries of adjacent lobules. Full-term stillborn infant. Injection of the pulmonary arteries. a–b Selected serial sections. Verhoeff 150.

TABLE 2 *Diameter and site of 15 pulmobronchial arteries demonstrated in pulmonary (PA) or bronchial artery (BA) injected lung specimens from 9 neonatal autopsy subjects*

| Specimen no. | BW (g) | Injection of | Diameter (μ) of pulmobronchial artery | Slt (lung lobe) |
|---|---|---|---|---|
| A 119 | 450 | PA | 25 | RL |
| | | | 75 | RL |
| | | | 100 | RL |
| A 23 | 930 | PA | 70 | LL |
| A 14 | 1750 | PA | 75 | LL |
| A 21 | 1985 | PA | 60 | LU |
| A 115 | 2670 | PA | 80 | RM |
| A 23 | 2720 | PA | 75 | LU |
| A 78 | 3270 | BA | 100 | RL |
| A 113 | 3550 | PA | 25 | RM |
| | | | 50 | LU |
| | | | 80 | LL |
| | | | 85 | LL |
| A 9 | 4200 | PA | 90 | RL |
| | | | 100 | RM |

and they are not accompanied by a bronchus at their points of origin. After entering an adjacent lobulus some of these branches can be seen to join an intra lobular bronchus of corresponding size A few of the abrupt branches give rise to pulmobronchial arteries or to arterial bronchopulmonary anastomoses. This feature will be discussed below The number of abrupt branches detected in the microangiograms varied in different areas and to some extent from specimen to specimen but in general four to six such branches are present between the ordinary bifurcations of the elastic pulmonary arteries. Their incidence is about the same in the immature premature subjects as in the full-term ones.

A few pulmonary arterioles leave their respective lobulus to supply either septal tissue or the pleura. Most of these run far from the hilus, in basal or lateral parts of the lung but some were observed in the pleura of the interlobar fissures near the hilus. The diameter of these extralobular branches of the pulmonary arteries, when distended with contrast ranges from 25 to 75 μ.

Pulmonary arterioles occasionally leave their lobulus and cross the interlobular septum to ramify as alveolar capillaries of an adjacent lobulus (Fig 2). This feature was not recognized in the microangiograms, but was encountered in a few specimens serially sectioned for the histologic examination of other features.

*Pulmobronchial arteries* In 9 subjects (24 per cent) a few intrapulmonary bronchial arteries originate either directly from elastic pulmonary arteries or from one of the abrupt branches mentioned above. These structures correspond to the *rami pulmobronchiales* or the *rami bronchiales arteriae pulmonalis* described in the litera-




Fig. 3

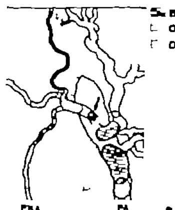



Fig. 3. Pulmobronchial artery (PBA, diameter 90 µ) originating from muscular pulmonary artery (PA); the point of origin indicated by corresponding arrows in b and The pulmobronchial artery divides int recurrent and concurrent branches before entering the bronchial wall, where these vessels assume the usual characteristics of bronchial arteries. They closely follow the peribronchial nerves (c–d). Pulmonary artery injected specimen from full term newborn infant. a. Microangiogram 17 b–d Selected serial sections from framed area in the microangiogram. Verhoeff 42. e. Diagram of the framed area in the microangiogram showing the pproximate levels of b–d

ture [4 15 16 19 57]. These vessels were recently dubbed "pulmobronchial arteries" by Wagenvoort & Wagenvoort [53] and that term has been adopted in the present study The diameter of the pulmobronchial arteries at their points of origin ranges from 25 to 100 µ (Table 3) After leaving their parent pulmonary artery they generally turn distally along the neighbouring bronchus but they also send recurrent branches along the same bronchus (Fig 3) In this way they resemble true side-to-side (H) anastomoses, but they could be distinguished from these since their recurrent branches gradually decrease in size towards the hilus, whereas the converse is true of the bronchial artery proximal to an H-anastomosis. The pulmobronchial arteries do not have precapillary communications with neighbouring branches of true bronchial arteries. Instead, they appear to substitute for these arteries along a few bronchi.

The mural structure of the pulmobronchial arteries, after they join the bronchial walls, cannot be distinguished from that of ordinary bronchial arteries They do not have the morphologic characteri tics of




Fig. 4. Focal bronchial artery supply of alveolar walls near the hilus. The contrast medium has reached pulmonary arterioles supplying adjacent areas (c, arrows), apparently via capillary intralobular communications. Bronchial artery injected specimen from premature infant (BW 1645 g). a. Microangiogram 21 b–c. Selected serial sections from framed area in the microangiogram. Verhoeff 52.

"Sperr"-arteries [15], i e their lumina are not narrowed by longitudinal intimal muscle bundles.

*Bronchial Arterial System*

*Basic pattern.* Apart from their ramifications in the bronchial walls proper the intrapulmonary bronchial arteries form the *vasa vasorum* of the elastic pulmonary arteries. They also supply septal tissue near the hilus as well as lymph nodes and peribronchial nerves. Pleural branches of the bronchial arteries are common, particularly near the hilus and at the interlobar fissures. None of these branches, however enter the pulmonary parenchyma to form anastomoses with the pulmonary arteries.

The inner diameter measured in the microangiograms of the main bronchial arteries in the hilus of the lung increases with fetal age and ranges from 1.5 to 500 $\mu$.

In the late fetal and neonatal lung none of the bronchial arteries have the wall structure of "Sperr"-arteries.

*Bronchopulmonary arteries.* In all but one of the aorta injected specimens, branches of different sizes (diameter $< 120\ \mu$) from the bronchial arteries leave the bronchial walls to ramify into capillaries of alveolar walls (Fig 4). Such "bronchopulmonary arteries [53] are particularly common in the medullary" zone of the lung and sometimes are surrounded by a narrow sleeve of lymphoid tissue at their point of entrance into the pulmonary parenchyma In places they substitute for the pulmonary artery along terminal bronchioles and here their wall structure cannot be distinguished from that of peripheral pulmonary arterioles (Fig. 5). Occasionally contrast from bron-




Fig 5. Detail of bronchial artery supplied area of the pulmonary parenchyma in full-term new born infant. The bronchial artery (BA) has substituted for the pulmonary artery along a termi nal bronchiole Bronchial artery injected specimen. Verhoeff 170.

chopulmonary arteries had reached pul monary arterioles of adjacent areas apparently via capillary communications (*Fig 4*)

The total volume of pulmonary paren chyma supplied by bronchial arteries though differing from specimen to speci men is consistently only a minute part of the lung

In a few instances a pulmobronchial artery originates not far distally from a bronchopulmonary artery indicating that the former substitutes for the latter by taking over the arterial supply of the bronchial wall (Fig. 6)

## *Arterial Bronchopulmonary Anastomoses*

True anastomoses between the bron chial and pulmonary arteries were demonstrated in six subjects by serial sectioning (16 per cent) The anastomoses are few in number with not more than two in each subject

Most of the anastomoses are of the side-to-side (H) type The mural structure of the transverse vessel of an H-anastomosis is generally the same as that of the contri buting bronchial artery In one of the H anastomoses, however there was a gradual change in the elastic pattern of the transverse vessel from bronchial artery type to pulmonary artery type The elastic com ponent of the vascular wall at the bronchial artery side consisted chiefly of an internal elastic membrane whereas towards the pulmonary artery there was a gradual increase in the amount of elastin fibres with the appearance of an external elastic membrane (Fig 7)

One anastomosis was of the end to-side type between a thin-walled arteriole derived from a bronchial artery and an elastic pulmonary artery (Fig 8)

Finally one intrapulmonary end-to-end anastomosis was found between a recur rent arteriolar branch of a bronchial artery and a pulmonary arteriole penetrating a lobulus

The diameter of the anastomoses ranges from 35 to 100 $\mu$ (Table 4) In the late fetal and neonatal lung these anastomoses do not have the morphological characteristics of "Sperr -arteries.

## Comment

It is generally agreed that the pulmonary artery branches together with the bronchi [3 14 15 17 40 52] but some

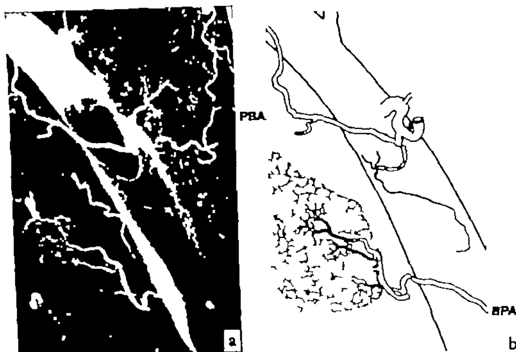



Fig. 6. A bronchopulmonary artery (BPA) crossing from lower right over partially filled branch of the pulmonary artery to ramify into the pulmonary parenchyma (*lower left*) is substituted for by pulmobronchial artery (PBA) originating near the centre. The pulmobronchial artery has concurrent as well as recurrent branches. Pulmonary artery injected specimen from full-term still-born infant. *a*. Microangiogram 12. *b* Diagram based on serial sections from the same area.

authors have noted the existence of abrupt muscular branches of the elastic pulmonary arteries, particularly in the medullary zone of the lung [7–9 11 57]. This study confirms that numerous such branches occur normally in the lungs of the newborn. It has further been stated that the intralobular pulmonary arteries are end-arteries within their respective lobules, with the exception of branches supplying the pleura [17 35]. This concept does not seem to be universally true since in the present series pulmonary arterioles were demonstrated that traversed interlobular septa to ramify into alveolar capillaries of adjacent lobules. Thus, the lobular pattern of the respiratory ducts and the alveoli is not in all places followed by the pulmonary arterial system.

It is evident from the present study that the bronchial arteries of the fetus and newborn in addition to the bronchial structures supply small areas of the pulmonary parenchyma proper. This feature was previously recognized by Küttner [19] by Konaschko [18] and by Cudkowicz & Armstrong [6]. These old observations were recently confirmed by Wagenvoort & Wagenvoort [53], who also suggested the term "bronchopulmonary arteries" for branches of the bronchial arteries ramifying into the capillary network of alveolar walls. In the present study there is evidence of capillary communications be-



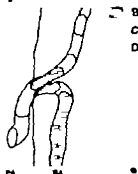




Fig 7 Arterial bronchopulmonary anastomosis of H-type (diameter approx. 100 μ) in bronchial artery injected specimen from full-term stillborn infant The anastomosis empties on top of the elastic pulmonary artery to the left (c). a. Microangiogram 7 b–d. The anastomosis in selected serial sections from framed area in the microangiogram. Not the gradual change in the mural elastic pattern of the transverse vessel. Towards the pulmonary artery there is an increasing component of elastic tissue in the vascular wall with the appearance of an external elastic membrane (c, arrows). Verhoeff 42. e. Diagram of framed area in the microangiogram showing the pproximate levels of b–d. PA = pulmonary artery BA = bronchial artery

tween the bronchopulmonary arteries and adjacent branches of true pulmonary arteries. This may be interpreted as a focal retention of the early fetal vascular pattern, in which the capillary plexus of the developing lung is connected with systemic as well as pulmonary arteries [4 5 24 28 47 54].

The double arterial supply of the capillary plexus of the early fetal lung is also said to favour the development of systemic-pulmonary arterial anastomoses [47 50 53 54]. In the present series, the incidence of such anastomoses was found to be 16 per cent In a few more subjects a considerable transmission of contrast from one arterial system to the other suggested precapillary communications although no arterial anastomoses could be demonstrated in the *microangiograms* or in the serial sections The stereo-microangiographic technique permits quite complete screening of the arterial systems of the lung but anastomoses may be hidden by close overlapping of pulmonary and bronchial arteries in the microangiograms or by being present near or at the borderline between two slices of lung tissue Thus, the true incidence of arterial bronchopulmonary anastomoses is probably some




Fig. 8. Arterial bronchopulmonary anastomosis of end-to-side type (35 μ) in bronchial artery injected specimen from premature infant (BW 1800 g). After crossing the wall of the bronchus, the bronchial artery (*right*) turns backwards and empties into the adjacent elastic pulmonary artery (*l*). *a*. Microangiogram 30. *b*. The point of anastomosis found in serial sections from framed area in the microangiogram. Verhoeff 180.

what higher than the one found in the present series.

Anastomoses between pleural branches of the bronchial arteries and peripheral pulmonary arteries have been described repeatedly in the human lung [15 19 23 50 51 57] These anastomoses are said to be of the end-to-end type and particularly prevalent on the mediastinal aspect of the lung and at the interlobar fissures [15 51 57] The pleural branches of pulmonary arteries observed in this study were largely found on the costal aspect of the lung where no bronchial artery supply of the pleura is said to exist [5 50 51]. Some pleural branches of the pulmonary artery however were not far from the hilus, at the interlobar fissures. The latter may very well be pleural arterial bronchopulmonary anastomoses, whereas those on the costal aspect of the lung probably represent a pulmonary arterial supply of the pleura. In aorta injected specimens, pleural branches of the bronchial arteries were frequently encountered, particularly at the interlobar fissures. None of these branches,

TABLE 4. *Diameter and site of 8 arterial bronchopulmonary anastomoses demonstrated in pulmonary artery (PA) or bronchial artery (BA) injected lung specimens from 6 neonatal autopsy subjects*

| Specimen no. | BW (g) | Injection of | Type of anastomosis diameter (μ) SS | ES | EE | Site (lung lobe) |
|---|---|---|---|---|---|---|
| A 73 | 1080 | BA | | 35 | | LL |
| A 16 | 1750 | PA | 100 | | | RM |
| A 13 | 150 | PA | 75 | | | LU |
| A 113 | [illegible]670 | PA | | | 30 | RM |
| | | | 75 | | | RM |
| A 2[illegible] | [illegible] | PA | 75 | | | LL |
| A [illegible] | 2[illegible]7 | BA | 100 | | | RL |
| | | | 75 | | | RU |

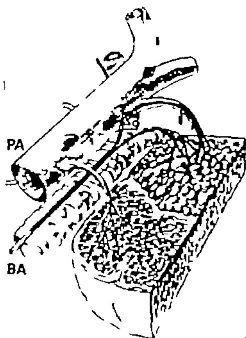
SS = side-to-side, ES = end-to-side, EE = end-to-end.

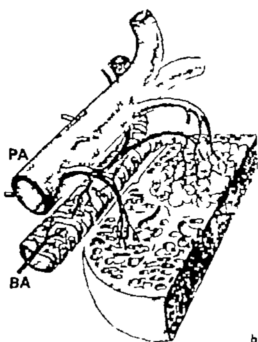
however were found to enter the pulmonary parenchyma to form anastomoses with pulmonary arterioles. There was then no conclusive evidence for the existence of pleural arterial bronchopulmonary anastomoses.

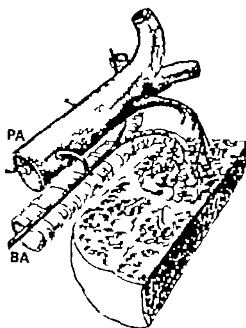
The disagreement among previous investigators concerning the normal occurrence of arterial bronchopulmonary anastomoses may in part be due to the different techniques employed. Serial sectioning has been used in studies confirming the existence of these anastomoses in the normal lung [15, 20, 51–54]. The failure to demonstrate the anastomoses by injection techniques in the normal adult lung [2, 6, 5, 37, 44, 48, 53] may be explained by their tortuous course in combination with the "Sperr"-artery structure gradually acquired by bronchial arteries and any bronchopulmonary anastomoses in postnatal age [10, 15, 20, 1, 38, 49, 50, 51, 54]. The incidence of anastomoses is further influenced by whether the pulmobronchial arteries are included in the concept of anastomosis or not. These arteries have been regarded as a form of arterial bronchopulmonary anastomoses by some authors [15, 5], but since they do not communicate on the precapillary level with adjacent branches of ordinary bronchial arteries, they should be distinguished from true anastomoses.

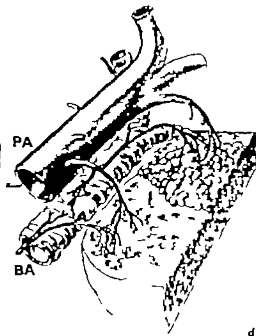
The abovementioned sources of error inherent in the microangiographic techni-

Fig. 8. Diagram showing some aberrations from the basic arterial pattern of the lung. Lobules in the "medullary" zone of the lung are supplied by ordinary branches of the pulmonary artery (PA) and by abrupt ones. The bronchial artery (BA) does not anastomose with the pulmonary artery nor does it leave the bronchial wall. This is the basic pattern. *b*. The bronchial artery is substituted for in the bronchial wall by a pulmobronchial artery originating from an abrupt muscular branch of the elastic pulmonary artery. The arterial bronchopulmonary anastomosis of II-type. *d*. The bronchial artery leaves the bronchial wall to ramify in the pulmonary parenchyma. It is substituted for by a pulmobronchial artery originating as an abrupt branch from the elastic pulmonary artery.


PA
BA
a


PA
BA
b


PA
BA


PA
BA
d

que used for the d monstration of anastomoses are naturally also involved in the searching for the points of origin of th pulmobronchial arteries The demonstration of these arteries is further influenced by whether the injection of contrast is made into the pulmonary or into th systemic arteries They are naturally most ea ily demon trated by injection of the pulmonary arteries In thi study only one pulmobronchial artery was found in the aorta injected pecimen and this particular vessel wa apparently filled via an adjacent 100 μ wide II-ana tomosi Other pulmobronchial arteries were certainly present in this part of the series though not vi ualized in the microangiograms Therefore it seem probable that the incidence of pulmobronchial arteries calculated from the pulmonary artery injected specimens only (8 of 17 subject 4 per cent) i closer to the true incidence than i the figure calculated from the whole material ( 4 per cent)

While the occurrence f pulmobronchial arteries was fairly uniformly distributed in the various birth weight groups true anastomoses were found only in subjects with a birth weight of 1680 g and more A gradually increa ing incidence of true anastomoses in the developing lung would be consi tent with recent observation in early infancy [4*]

The diameters of the pulmobronchial arteries and of the true anastomoses (Table 3 and 4) were mea ured on vessel injected and often distended with contrast medium and cannot therefore be regarded as a reliable index of their functional capacity Since however their diameter does not seem to exceed 100 μ and since both the pulmobronchial arteries and the anastomoses are few in number and apparently not even constantly present their functional significance in the lat fetal and neonatal lung is probably low [*0 52]

## Conclu ion

The ba i intrapulmonary arterial pattern i that of two essentially separate arterial systems one supplying the pulmonary parenchyma *proper and the other* the bronchial structures Aberration from this ba ic pattern normally occur in the human fetu and newborn in the form of

(a) pulmobronchial arteries which substitute for the true bronchial arteries along a few bronchi

(b) f cal bronchial artery supply of the pulmonary parenchyma proper through "bronchopulmonary arteries" and

(c) arterial bronchopulmonary anastomoses

These aberration seem to occur haphazardly but may be interrelated a indicated in Fig 9

## Summary

The pulmonary and bronchial arteries of 67 lungs from 37 immature premature and full term newborn infants were studied by microangiography and serial sectioning. Intrapulmonary arterial bronchopulmonary anastomoses were demonstrated in small numbers in 10 per cent of the subjects. Pulmobronchial arteries, i e intrapulmonary bronchial arteries originating from pulmonary arteries were found in 24 per cent of the subjects In specimens injected into the bronchial arteries, these were constantly found to supply small areas of the pulmonary parenchyma proper particularly in the medullary zone of the lung In su h areas branches from bronchial arteries substitute for the pulmo-

nary artery along occasional terminal bronchioles. On the other hand, a bronchial artery leaving its usual position in the bronchial wall to ramify into the pulmonary parenchyma may be replaced in the bronchial wall by a pulmobronchial artery. These features can therefore be interpreted as probably interrelated focal aberrations from the basic arterial pattern of the normal lung, which is that of two essentially separate arterial systems.

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# The Excretion of Delta Aminolaevulinic Acid by Children

by DONALD BARLTROP

Delta-aminolaevulinic acid (ALA) is well known to be an intermediate in the synthesis of haem and small mounts are normally excreted in urine. An increased excretion of this substance occurs in porphyria [3] and in lead poisoning It has been suggested that the excretion of ALA by animals and human adults is the most sensitive indicator of excessive amounts of lead in the body [5]. Several attempts have been made to apply this information to the diagnosis of unsuspec ted lead poisoning in large groups of chil dren at risk, but the results have so far been disappointing [4 14]. The standards adopted for the upper limit of normal excretion of ALA by children have in the past varied widely in different reports –6 mg/24 hours [11], 4.8 mg/l [13], 30.3 mg/l [8].

Since the excretion of ALA may be closely related to the non-skeletal or metabolically active lead status of the body it should be recalled that lead poisoning of children differs from that of adults in its tendency to well-defined age (1–5 years) and seasonal limits (J ne–September). This study ha attempted to define the excretion of ALA by a large group of hospital in patient children who had no evidence of bnormal exposure to lead.

This paper is based on data forming part of thesis for the degree of M D submitted to the University of London.

## Methods

The children studied were those admitted to Princess Louise (Kensington) Hospital for Children, London, during an 11-month period. Base data were recorded on admission on a *pro forma* together with details f symptoms suggestive of lead poisoning and details of exposure to lead containing substan ces. The racial group f both parents and the social class of the family were determined.

A 24-hour urine collection was attempted for each child but those admitted for periods of less than 24 hours or for genital or inguinal surgery were excluded. In young children an adhesive disposable urine collector bag was pplied and connected throughout the col lection period t an aspiration device. Urine volumes were recorded within three hours of the completion of the collection and ali quots were then frozen at −20°C. An early morning specimen of urine and sample of venous blood were btained after the 24-hour urine collection.

Urine ALA and porphobilinogen were determined by ion-exchange chromatography using a modification f the method of Mau zerall & Granick [7]. Urine coproporphyrin was determined using the semiquantit t ve method of Benson & Chisolm [2] with com mercial mesoporphyrin IX standards Blood and urine lead determinations were made after preliminary wet-ashing by means f the colour reaction with alkaline dithizone Haemoglobin was determined photoelectri cally as xyhaemoglobin and basophilic stippled cells were counted under dark ground microscopy [6].

## Results

Successful 24 hour urine collections were obtained from 339 of the 401 children admitted during the period of the study. They were aged 0–12 years and comprised 213 males and 126 females.

The excretion of ALA by the whole group of children was found to range from 0–6.5 mg/24 hours. 9?% of the values lay between 0.08–4.37 mg/24 hours. The mean value for the whole group was 1.04 mg/24 hours. The distribution curve was plotted for *0.2 mg increments* and was found to be positively skewed (Fig. 1).

The excretion of ALA by different age groups was determined for each of the first 12 years. The mean excretion rose progressively from 0.3? mg/24 hours in the first year of life to 1.09 mg/24 hours in the fifth year, but after this age the mean value tended to plateau. The mean values, together with the range, 5th and 95th percentiles are illustrated in Fig. 2.

The excretion of ALA per unit body weight was determined and the mean value was found to vary between 0.0?–0.10 mg/24 hours/kg body weight with an overall mean of 0.08 mg/24 hours/kg body weight. There was no correlation with age although children excreting more than 0.2 mg/24 hours/kg body weight were confined to the 0–6-year age groups. Details are illustrated together with the 5th and 95th percentiles in Fig. 3.

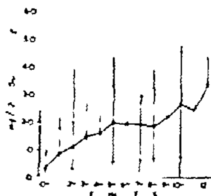


Fig. 2. The mean, range, 5th and 95th percentile limits for the 24-hour urine ALA excretion by 339 children at different ages.

The mean excretion of ALA per unit body weight was determined for bi-monthly periods throughout the year. A cyclical variation in the mean excretion of ALA took place with maximum values in the period January–April and minimum values in July–August (Table 1). The difference between the extreme values was significant (January–February, $p = 0.02$; March–April, $p = 0.0?$).

During the study a six-year-old male child was found to have excreted 13.4 mg ALA/24 hours and analysis of a second specimen of urine showed that it contained porphobilinogen 1.4 mg/100 ml and coproporphyrin 0.5 μg/ml. The findings in this case of acute intermittent porphyria were excluded from the data in this paper and have been fully reported elsewhere [1].

No correlation was found between the excretion of ALA and the sex, race, social class or diagnosis of the children studied.

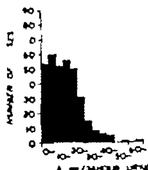


Fig. 1. The distribution of 339 children according to the 24-hour urine ALA excretion.

TABLE 1 *The mean 24-hour urine excretion of ALA by 339 children at different periods of the year*

| Period | Number of children | Mean ALA excretion, mg/24 hours/kg body weight |
|---|---|---|
| January–February | 52 | 0.094 |
| March–April | 22 | 0.090 |
| May–June | 79 | 0.083 |
| July–August | 74 | 0.076 |
| September–October | 51 | 0.080 |
| November–December | 61 | 0.084 |

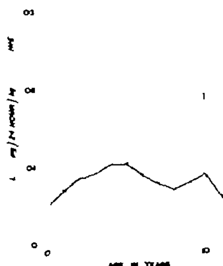



Fig. 2. The mean, range, 5th and 95th percentile limits for the 24-hour urine excretion of ALA per kilogram body weight by 339 children at different ages.

There was no correlation with the basophilic stippled cell count, haemoglobin concentration, occurrence of coproporphyrinuria or the 24-hour urine lead excretion.

The blood lead concentration was determined in 329 of the children and a weak correlation with the 24-hour urine ALA excretion was found. The correlation coefficient was significant only at the 1% level however and the residual variance about the regression was too large for the relationship to have any practical predictive value.

## Discussion

The fivefold increase in the mean excretion of ALA during the first six years of life from 0.35 to 1.00 mg/24 hours emphasizes the need for standards for different ages of children. Upper limits of normal for the 24-hour excretions of ALA by adults have been given as ... mg [10] ...5 mg [0] and ...6 mg [1 ], and assuming body weights within the normal range these represent considerably lower rates of excretion than those reported here. This difference could represent a greater rate of porphyrin metabolism in children compared with adults, however there was no evidence that the rate of ALA excretion per unit body weight fell with increasing age in this study but the rate remained fairly constant irrespective of age (Fig. 2).

The absence of any correlation between the 24-hour urine ALA and lead excretions is in contrast to the work of Haeger-Aronsen [5] who described an almost linear relationship between the concentrations of these substances in the urine of adults exposed to lead. The children described in this paper had not been abnormally exposed to lead, however, and the range of blood lead values observed in 329 of them was small (90% of the values in the range 0.07–0.53 μg/ml whole blood, mean 0.21 μg/ml).

The seasonal variation in the excretion

of ALA wa a *finding* that would probably have been overlooked but for the seasonal variation in lead poisoning Since the excretion of ALA was at a minimum in the period July August and the magnitude of the variation was small it is unlikely that this would obscure the diagnosis of lead poisoning in children which is at a maximum during this period

*0.0-6.5* mg 0.. of the values were in the range 0.08-4.30 mg/24 hours. The mean excretion of ALA per unit body weight was 0.08 mg/kg body weight/24 hours and this value did not vary with age A seasonal variation in the mean excretion of ALA per unit body weight was found with maximum values in the winter months and minimum values in the summer

## Summary

Twenty four hour urine specimens were collected from 339 children aged 0 1* years admitted to hospital over an 11 month period The mean excretion of ALA per 24 hours was 1.01 mg with a range of

## Acknowledgement

I am indebted to Dr P Richard for gift of ALA and porphobilinogen. The blood and urine lead estimations were made by Miss J A Peel. Mr W Sheppard estimated the urinary coproporphyrins and performed the basophilic stippled cell counts.

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# Respiratory Syncytial Virus Infections in Hospitalized Children

## *Evaluation of the Virus Isolation and Complement Fixation Techniques in the Virological Diagnosis Clinical and Epidemiological Characteristics*

*by* BO BERGLUND and CARL-HENRIK STRÅHLMANN

For isolation of respiratory syncytial (RS) virus in tissue cultures various continuous cell lines or primary trypsinized cells of human or monkey origin have been employed. The U cells used for propagating the RS virus [1 2, 3, 6 11] constitute continuous line of human amnion, originally derived from Utrecht, Holland. It has been passaged up to now uninterruptedly for 7 years in the laboratories in Finland. However the susceptibility of these cells to RS virus has not been more extensively evaluated.

In association with RS virus infection in infants the frequency of signs of middle ear inflammation has been reported as varying from 5 to 50 % [1 4 5 8].

The presence of RS virus, however in the middle ear exudates in cases of otitis media, as far as known, as yet has not been established.

Clinical signs and symptoms of RS virus associated respiratory disease in children in Finland have been recorded previously during an outbreak which took place at the end of 1963 in the cities of Turku and Helsinki and continued some way into the next year [1 4]. The causative agent of this outbreak was demonstrated to be antigenically closely related to the Randall strain of RS virus [2]. However clinical signs and symptoms of children in Finland with RS virus infection due to strains antigenically different from the Randall strain have not been recorded.

This report describes the usefulness of U cells for isolating RS virus, and the efficiency rate of the isolation method used in detecting infections, as compared to the complement fixation (CF) test Signs and symptoms of disease associated with a strain of RS virus differing antigenically from the Randall virus are described, and data is presented concerning the first isolations of RS virus from the middle ear exudates of children with acute respiratory disease due to this virus.

The study was performed during an outbreak of respiratory illness caused by RS virus, in Turku, Finland extending from early April to late June, 1965

This study was supported by the National Research Council for Medical Sciences.

## Material and Methods

### *Subjects*

The series consists of virtually all children under 6 years of age admitted with acute respiratory disease to the wards of the Hospital for Infectious Diseases, Turku, in May

and June 1965. The children studied numbered 60. In taking the *medical history* attention was called to the date of onset of the respiratory symptoms, and to the place at which the children stayed during the day time in cases where the parents were working outside the home. The study does not include children institutionalized throughout the twenty-four hours.

After hospitalization the children were subjected to a careful physical examination and daily medical observation, as well as to an x-ray examination of the chest. The clinical diagnosis was made at the end of the hospitalization period. Rhinitis and/or cough indicated an upper respiratory infection, whereas children with additional rhonchi and wheezing, or musical râles were diagnosed as having bronchitis. Signs of breathing difficulties, such as cyanosis, tachypnea, prolonged expiration or retractions indicated bronchiolitis, crepitant râles and/or apparent x-ray evidence of consolidation of the lung parenchyma pneumonia.

*Isolation of virus*

The U cells used for virus isolation were originally supplied by Dr Doorschodt, Hygienisch Laboratorium der Rijks Universiteit, Utrecht, Holland. The composition of the growth and maintenance media of the cells has been described elsewhere as well as the handling and observation of the cultures *before and after inoculation* [3]. On admission or the following day cell culture tubes were directly inoculated with throat swabs, a separate cotton applicator being used for each tube. The fluid remaining in the cotton was pressed out by rubbing the swab along the inner surface of the tube. Twenty four hours after the first sampling 2 more throat swabs were obtained from almost all children, and the swabs used for inoculating in the same way of cell culture tubes. In the case of syncytial cytopathic degeneration in a culture—this appeared usually on day 4—sampling was *immediately continued*, isolation specimens being obtained each day until the child left the hospital.

*Specimens of the middle ear exudate for* isolation of virus were obtained in a few cases of exudative otitis media according to a technique reported elsewhere [3].

*Identification of RS virus*

Isolates producing syncytial degeneration were identified as RS virus by a CF technique *using paired sera from children with* confirmed RS virus infection. This technique has also been described in a previous paper [4]. If the convalescent serum diluted to contain 4 antibody units fixed complement completely at an antigen dilution of at least 1/8 but the equally diluted acute serum did not fix complement with the 1/8 diluted antigen, this indicated that the isolate was RS virus.

*Identification of other viruses*

Adenoviruses were identified according to a CF method described elsewhere [ ]. Herpes virus was identified by using properly diluted immune serum from a rabbit with herpetic keratoconjunctivitis according to a *neutralization technique indicated elsewhere* [7].

*Collection of serum specimens*

The 1st specimen was obtained at admission, or the next day: the 2nd one 10–14 days later. From children under 6 months of age a 3rd specimen was taken about 3 weeks after the 1st one.

*Determination of CF antibodies in serum specimens*

The Randall strain, cultivated in U cells grown and maintained as described previously [3], was used for the preparation of RS virus CF antigen. The cultures were harvested at the time of complete degeneration of the cell sheet, frozen at $-70°C$ and thawed 10 times, after which the material was *titrated against 8 antibody units* of a pool of early convalescent RS virus immune serum from 4 children. Eight units of antigen and 1–2 units of complement were employed in the tests. The RS virus CF antibody titration was carried out by a slightly modified Sever micro method described previously

[3]. The titer was the highest initial dilution of serum giving no more than a 1 + hemolysis as determined by visual estimation.

The above method was also used for determining the CF antibody titer of following agents: Influenza A, B, parainfluenza 1 adeno- and cytomegalovirus.

## Results

*Virus isolations*

When syncytial changes appeared in a throat swab inoculated culture the collection of isolation specimens was immediately repeated, as outlined in the section of Material and Methods, and continued until the child was leaving the hospital. Twenty seven children were found to excrete virus in their throat secretions and over *00 specimens were thus gathered from these children and cultured for virus. It was self-evident that the chances for obtaining a positive virus isolation diminished as the disease developed into the convalescent stage Table 1 shows the frequency of positive RS virus isolations at various stages of the respiratory disease of 24 RS virus infected subjects for whom reliable information concerning the date of onset of their respiratory symptoms and signs were available As expected, the relative number of positive isolation specimens wa highest (82 %) during the first week after onset of the respiratory disease Then it gradually decreased, as the disease progressed, only a few specimens yielding virus in the 4th week of illness. In one case —that of a year-old boy—the interval between two positive specimens was no less than 22 days.

As mentioned above, 4 isolation specimens were obtained in almost all instances from each child studied and the specimens used for inoculation of equally many cell culture tubes during two subsequent days following admission. All subjects found positive by virus isolation harboured virus in their throat secretions already on the first day of specimen collection. It would seem that this means that repeated sampling during subsequent days is unnecessary On the other hand, it was found necessary even at the acut stage of the disease to take throat swabs for the inoculating of more than one culture· The isolation results obtained at the 1st sampling showed that in 14 % of the virus-positive cases only one tube was positive and in 86 % both tubes. This implies that 14 % of the RS virus infections could have remained undetected by virus isolation had, altogether only 1 throat swab been taken from each child shortly after admission for the inoculating of only 1 cell culture tube At subsequent samplings, where positive isolations were obtained, the com

TABLE 1 *Frequency of RS virus isolations in 24 children under 6 years age at various phases of their respiratory disease, associated with this virus*

| Time interval in days after onset of respiratory symptoms | No. of positive specimens | No of specimens taken | Percentage of positive specimens |
|---|---|---|---|
| 0–7 | 54 | 66 | 82 |
| 8–14 | 35 | 71 | 49 |
| 15–21 | 8 | 38 | 21 |
| 22–*6 | 2 | 18 | 11 |
| Total | 99 | 193 | 51 |

All specimens from _1 children with one or more positive isolations are included in the Table, as well as 9 virus-negative specimens from 3 subjects who were diagnosed as having RS virus infection by serological procedures only

TABLE 2. *Time of appearance of first signs of RS virus induced syncytial degeneration in throat swab inoculated cultures*

| Observation period in days | No. of cultures positive |
|---|---|
| 1 | — |
| | — |
| 3 | 2 |
| 4 | 24 |
| 5 | 11 |
| 6 | 11 |
| 7 | 10 |
| 8 | 4 |
| 9 | 3 |
| 10 | 4 |
| 11 | |
| 1 | 1 |
| 13 | — |
| 14 | 1 |
| Total | 73 |

panion throat swab failed to yield virus in no less than 30 of the cases. These results indicate that little is to be gained from inoculating more than two cultures each with one of two specimens, at the acute stage of the disease but that there is an increasing need to take more than 2 isolation specimens preferably on several occasions, in the later course of the disease.

Table 2 shows the time between inoculation and the appearance of first signs of the syncytial cytopathic effect in 73 cultures inoculated with the same number of throat swabs obtained within 1 to 3 days after hospitalization. In no less than 24 cultures (33 ) the syncytial changes became apparent after an observation period of 4 days. In a considerable number of the cultures, however syncytial degeneration was not observed until 5, 6 or 7 days or even longer after inoculation.

In one case RS virus and adenovirus were isolated from the same specimen. At first syncytial changes were prevalent, but after a few subsequent passages they were replaced by fast round-cell degeneration due to adenovirus. Adenovirus was recovered from a total of three subjects, herpes virus from one.

RS virus was isolated from the middle ear exudate of two children 7 months and 10 months old, with bilateral otitis media characterized by redness and bulging of the ear drum. At the time of puncture both were excreting RS virus in their throat secretions. One had bronchitis, the other pneumonia. The exudates withdrawn were not cultured for bacteria, but it appears probable that the comparatively strong signs of otitis media noticed in both these cases were due to bacteria. The role played by the RS virus in causing signs of middle ear inflammation cannot be evaluated for the present.

*The efficiency of the virus isolation method and the CF test in detecting RS virus infection*

Table 3 provides information concerning the efficiency rate of the isolation method used for isolating RS virus. It shows the virus isolation frequency in children of various ages with serologically confirmed RS virus infection. The specimens of one of the children (in the age group 3 - 6 months) listed in the table unexpectedly proved negative when tested for virus. However it appears likely that the throat swabs were obtained at the convalescent phase of infection, which might afford an explanation for the unsuccessful isolation attempts in this case. On the other hand, the failure to isolate virus from two other subjects, also listed in the table may re-

TABLE 3. *Frequency of RS virus isolations in 22 children with 4-fold or greater increase in CF antibody to this virus*

| Age | Average time in days between collecting paired blood specimens | No. of isolation positive children | No. of isolation negative children | No. of children tested |
|---|---|---|---|---|
| < 3 mo. | | | | — |
| 3 mo. – < 6 mo. | 12 | 4 | 1[a] | 5 |
| 6 mo. – < 1 yr | 10 | 5 | — | 5 |
| 1 yr – < 2 yr | 28 | 4 | — | 4 |
| 2 yr – < 6 yr | 13 | 6 | 2[b] | 8 |
| | Total 16 | 19 (86%) | 3 | 22 |

[a] Three months old. The throat swab specimens were obviously obtained at the convalescent phase of infection: CF antibody response 1/8 – 1/32.

[b] One was aged 5 years 3 months, his CF antibody response: < 1/4 – 1/16. The other was 5 years 3 months, her CF antibody response: < 1/4 – ≥ 1/256.

flect increasing difficulties in isolating RS virus from children of 5 years or over. The over-all isolation rate in all children with serologically confirmed RS virus infection was 86%. This percentage would presumably have been higher if any of the few under 3-month-old study subjects had developed a serologically significant response to RS virus and that it would have been possible to include their isolation results in the table. As previously shown the likelihood of recovering RS virus is obviously greatest among children under 3 months old [4].

Table 4 affords information on the efficiency rate of the CF method used for detection of RS virus infection. As shown, three children under the age of one failed to develop detectable diagnostic (4-fold or greater) antibody increases in response to their RS virus infection. In contrast, all children of 1 year or over did respond to this virus with a diagnostic rise in CF antibody. Thus the serologic response varied with age. The overall efficiency rate of the CF test (86 %) was the same as for the isolation method. However, sera from all children under 6 months of age obtained in the late convalescent phase of infection were included in the tests. This was likely to increase the likelihood of detecting infection by the CF technique in children in this youthful age group [1, 4, 9].

*CF antibody responses against agents other than RS virus*

Four subjects, two of whom had RS virus infection, exhibited a 4-fold or greater rise in CF antibody to adenovirus, and three, two of whom also had RS virus infection, showed a 4-fold rise in the cytomegalovirus CF antibody titer. No other diagnostic antibody increases to the agents employed in the tests were observed.

*Clinical picture of respiratory illness in children with serologically confirmed RS virus infection*

Table 5 provides information on the distribution of RS virus infected children according to age and clinical diagnosis. It shows the predominance of lower respira

TABLE 4 *Frequency of 4 fold or greater rise in CF antibody titer to RS virus in 22 children positive by RS virus isolation*

| Age | Average time in days between collecting paired blood specimens | No. of  hildren positive by CF technique | No. of children negative by CF technique | N of  hildren tested |
|---|---|---|---|---|
| < 3 mo. | 33 | — | 1 | 1 |
| 3 mo. – < 6 mo. | 15 | 4 | 1[b] | 5 |
| 6 mo. – < 1 yr | 11 | 5 | 1 | 6 |
| 1 yr – < 2 yr | 8 | 4 | — | 4 |
| yr – < 6 yr | 9 | 6 | — | 6 |
| Total | 15 | 19 (86%) | 3 | 22 |

This child was only 1 month old.
[b] Only 3 months old. Interval between blood specimens 1 days. Rise in CF antibody titer from < 1/4 t 1/4
Eleven months old. I terval between blood specimens 13 days. N plausible explanation for the absence of CF ant body response (titer < 1/4 < 1/4).

tory illnesses in children in the younger age groups but the occurrence of pneu monia as well as upper respiratory tract infections, in the older age groups. Bronchioliti as defined in the present study did not occur at all in children of one year or over

Table 6 summarizes the symptoms and signs of 34 children with RS virus infection. The comparatively high frequency of otitis media (38 ), may partly reflect the fact that this complaint wa in some cases one of the most important reasons for hospitalization.

*Epidemiological f ndings*

Table 7 indicates that roughly two thirds of all children under 6 years of age admitted to the wards of the hospital for acute respiratory illnesses during the *

TABLE 5 *Distribution according to age and diagnosis of 34 children with RS virus infection*

| Age | No. of childre | | | | |
|---|---|---|---|---|---|
| | Pneumonia | Bronchiolitis with pneumonia | Bronchiolitis | Bronchitis | Upper respiratory infection |
| < 3 mo. | 1 | 1 | — | — | — |
| 3 mo. – < 6 mo. | 4 | 1 | 1 | — | 1 |
| 6 mo. – < 1 yr | 1 | | 1 | | 1 |
| 1 yr < yr | 3 | — | — | 3 | — |
| yr – < 6 yr | 3 | — | — | 5 | 4 |
| Total | 1 | 4 | 2 | 10 | 6 |

Virus isolation from throat swabs, and/or 4-fold or greater rise in CF antibody titer
[b] Probably convalescent phase RS virus infection in a 3-month-old child with negative virus isolation, but rise in CF t ter from 1/8 t 1/32.

TABLE 6. *Respiratory symptoms and signs of 34 children under 6 years of age with RS virus infection*

| Symptoms and signs | No. of children |
|---|---|
| Rhinitis | 34 |
| Cough | 34 |
| Rhonchi | 22 |
| Crepitant râles | 15 |
| Otitis media | 13 |
| Musical râles | 11 |
| Dyspnoea | 9 |
| Wheezing | 5 |
| Retractions | 4 |
| Tachypnoea | 3 |
| Cyanosis | 2 |
| Pharyngitis | 2 |
| Sinusitis | 2 |
| Laryngitis | 1 |

Virus isolation from throat swabs, and/or 4-fold or greater rise in CF antibody titer

month study period suffered from RS virus infection. The proportion of RS virus and other infections was almost the same in the older age groups, but among the younger children RS virus infections predominated.

TABLE 7. *Distribution according to age and virologic findings, of children with acute respiratory disease.*

| Age | Children with RS virus infection | Children with no RS virus infection |
|---|---|---|
| 3 mo. | 2 | 1 |
| 3 mo. < 6 mo. | 7 | — |
| 6 mo – 1 yr | 7 | 10 |
| 1 yr < 2 yr | 6 | 5 |
| 2 yr – < 6 yr | 12 | 10 |
| Total | 34 | 26 |

Virus isolation from throat swabs and/or 4 fold or greater rise in CF antibody titer

No RS virus isolated. CF titer < 1/4 in paired sera of 23 subjects. CF titers of 3 subjects 1/32 1/32 1/16 1/16, and 1/64 1/32 respectively

Table 8 indicates that the proportion of day nursery residents was higher among children with RS virus infection (44 %) than among those hospitalized during the same period for illnesses due to other respiratory agents (8 %). The difference is statistically significant ($\chi^2 = 7.23$ $p < 0.01$), but is difficult to interpret since some of the children were admitted for social reasons, and thus it is possible that the day nursery children obtained entrance to the hospital more easily than the others. In any case it is an interesting observation and might suggest that on average RS virus infections, are more common in day nursery (and nursery) groups than among other children.

## Discussion

The results show that the isolation method and the CF test were equally sensitive as means of detecting RS virus infection. The results obtained with the CF test, in the present study however apparently reflected the maximum or nearly the maximum capacity of this test. The chances for detecting CF antibody increases were enhanced by examining for CF antibody additional post-convalescent phase sera from all children under 6 months with confirmed slow and weak antibody response to RS virus infection nd by including not less than 8 antigen units in the tests. In contrast the result achieved with the virus isolation method pparently did not indicat the maximum efficiency rat of this method

To obtain the optimal results from the direct isolation method used here it seems important to inoculate not less than 2

TABLE 8 *Residence of 5? children under 6 years of age before treatment in hospital for acute respiratory disease during an outbreak associated with RS virus*

| Place the child stayed in the day time while parent working | Children with RS virus infection[a] | | Children with no RS virus infection[b] | |
|---|---|---|---|---|
| | No. | | No. | |
| At home | 1 | 34 | 18 | 7 |
| Day nursery | 14 | 44 | | 8 |
| In another family | 3 | 9 | 4 | 16 |
| Kindergarten | 3 | 9 | 1 | 4 |
| Total no. of children | 32 | | 23 | |

[a] Virus isolation from throat swabs and or 4 fold or greater rise in CF antibody titer

[b] No RS virus isolated. No rise in CF titer

culture tubes each with a separate swab containing fresh throat secretion obtained at an early stage of the disease not later than the first week after onset of symptoms. In some instances one of the two throat swabs failed to yield virus even at an early stage of the disease. The reason for this is unclear. Probably it is a matter of technically unsuccessful sampling resulting in a low content of virus in the swab not sufficient to infect the inoculated culture. Therefore it seems desirable to use a sufficient amount of throat secretions as inoculum and, after immersing the swab into the culture fluid, to press out the fluid remaining in the cotton carefully by rubbing the swab against the inner surface of the culture tube.

One of the advantages of the isolation method used was the comparatively rapid appearance of the syncytial cytopathic effect in most instances on day 4 or within 1 week after inoculation, thus making possible an early presumptive diagnosis of RS virus infection. The appearance of detectable syncytial cell degeneration was considerably accelerated by changing the cultures to a fresh maintenance medium 1 or 2 days after the inoculation.

The RS virus outbreak in 1965 when the present study was carried out was preceded by another one in 1963. The results of the study carried out in 1963 have been published elsewhere (4). Both studies were carried out under similar conditions, the subjects being each time the children under 6 years of age admitted to the wards of the city's Hospital for Infectious Diseases during a month period. The results of both these studies are thus, to some extent comparable to each other. A comparison of the clinical results shows that RS virus-associated pneumonia and bronchiolitis were encountered in the children during the outbreak in 1963 as well as in 1965. As already stated in a previous paper [ ] the 1963 strain was completely—while the 1965 strain was incompletely—neutralized by immune serum prepared against the Randall strain of RS virus. The different antigenic composition of the two RS virus strains probably responsible for either of

the two outbreaks thus, on the whole did not seem to influence the symptomatology of the infections. The 1963 study included 87 children with acute respiratory disease of whom 27 (31 %) had RS virus infection the 1965 study had correspondingly 60 children of whom 34 (57 %) had RS virus infection. The 27 RS virus infected subjects of the former study were, on average younger and exhibited signs of more severe illness than did the 34 RS virus infected subjects of the latter one The comparison seems to suggest that agents causing severe respiratory illness, other than RS virus, were more prevalent at the time of the 1963 study and, therefore the need for hospitalization more pressing then than in 1965 at the time of the present study

As reported elsewhere, RS virus is easily transmitted among institutionalized children during outbreaks. Kapikian *et al.* [5] reported 91 % infection rate in infirmary children, Sterner *et al.* [10] a 100 % infection rate in nursery children. During an outbreak of respiratory illness in a nursery in Finland [2], RS virus was isolated from 10 of 1 children tested (83 %) In the light of these observations it would seem understandable that the group infected with RS virus in the present study was composed of proportionally more day-nursery children than the group infected with respiratory agents other than RS virus. However as mentioned under the heading Results" it is possible that social indications contributed to hospitalization of some of the day-nursery children. The results therefore only suggest but do not indicate with certainty that RS virus infections are on verage more common mong nursery children than among other children groups.


## Summary

The isolation results obtained after direct inoculation of cell cultures of a continuous line of human amnion (U cells) with throat secretions from 4 children under 6 years of age with RS virus infection indicated that virus was recoverable in 82 % of the specimens during the 1st week of illness, this percentage gradually decreasing to 11 % in the 4th week of illness. Satisfactory isolation results were obtained by inoculating culture tubes, each with one of two swabs on the day of admission, or next day Repeated sampling later on did not improve the results. Inoculation of the cultures with acute phase specimens resulted in the appearance of definite easily recognizable syncytial degeneration in 33 % of the cultures on the 4th day after inoculation, and in 80 % of the cultures within 1 week. The importance of obtaining a proper inoculum and changing the inoculated cultures to fresh medium is stressed.

A comparison of the isolation method and the CF test showed that the efficiency rate of both techniques in detecting RS virus infection was the same 86 %, but that the former afforded certain advantages not shown by the latter

The clinical diagnoses of RS virus infections in children of various ages are recorded, as well as signs and symptoms of their associated disease

Epidemiological aspects of RS virus infections are discussed, and a report is given on successful isolation of RS virus from middle ea xudates of two children with otitis media.

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# Rubella Immunity as Related to Age and History of Overt Disease

*by* ROLF LUNDSTRÖM, ARNE SVEDMYR, LARS HAGBARD and KURT KAIJSER

The development of tissue culture techniques for the cultivation of rubella virus and for the detection of neutralizing antibody in patients with rubella [10 18] followed by the demonstration that rubella could be transmitted only to subjects who did not have neutralizing antibody individuals with pre-existing antibody being immune [1 -, 5 6, 11 12, 16] made it possible to determine the frequency of susceptibility to rubella in different populations.

Sever *et al.* [15] found that 18 of 600 women 14-44 years old from different parts of the United States did not have neutralizing antibody against rubella virus, a higher incidence of susceptibility demonstrated in the negro (21 ) than in the white women (14 .). A sample from Honolulu, Hawaii, revealed 44 % without antibody The frequency of antibody was not significantly associated with histories f rubella. Dodgeon *et al.* [3] found 9 seronegative specimens among 50 cord bloods (18 ) from a London obstetric hospital and 6 seronegative among 25 cord bloods ( 4 .) from Kampala, the capital f Uganda. Further studies by Sever *et al.* [13] showed that 60 % of 223 pregnant women in Hawaii had no antibody to rubella. Nagayama *et al* [9] found 35 % seronegative among 183 serum specimens from pregnant women 18-40 years of age in Fukuoka city and its suburbs in Southern Japan.

Givan *et al.* [4] studied small groups of females of different age in Toronto, Canada, and found neutralizing rubella antibody in about 10 % of children up to 4 years increasing to about 90 % in those 35-40 years old. These tests were performed with a serum dilution of 1 6. It was also reported that the incidence of females with higher antibody levels (a titre of at least 24) reached a peak of more than 50 % in the age group 15-19 years falling to 20 % in those 35-40 years old. Sever *et al.* [14] determined the frequency of antibody in serum specimens from 268 individuals in Montgomery County Maryland, U.S.A., collected in 1957 The frequency of serological immunity increased from approximately 35 . in children 1 10 years of age to 85 in the group 16 years and older

Knowledge of the distribution of antibody against rubella is essential for estimations of the susceptibility to the disease thus providing the basis for an evaluation of th need for specific prophylaxis A cordingly the frequency of rubella anti

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body was determined in a Swedi h femal population selected from th inhabitant of the city of E kil tuna and its surround ings Th i is an indu trial area of Central Swed n with al out 60 000 inhabitants

## Material an l Method

### Collecti n of blo d spe imen

Blood pecimens were collected in August through December 1965 from 315 females f different age groups, each gro p consisting of 35 ind viduals. The youngest group was represented by the cord bloods of newborn infants, born by mothers 16–43 years of age (mean 27 years) The year and 5-year groups belonged to a lientele of a Child Welfare Center the 10-year and 15-year groups consisted f school children, the other gr ups were recruited from w men attending a Maternity Welf re Center were lying in patients of the maternity lepartment or were out patients of the gynecological linic A few samples were obtained from women attending a general practice The study material was chosen according to age only and regardless of hist ry of rubella. After collection the blood was stored overnight at 4°C The serum was then separated and tored at 60 C before transport with dry ice t the lal oratory where t was kept at −20°C until tested.

### Tissue cultures

The continuous cell line RK 13 of rabbit kidney shown by McCarthy and coworkers [8] to be s table for rubella virus work was obtained from Dr J. A. Beale Glaxo Labora tories Ltd. It was freed from a mycoplasma contaminant through the use f kanamycin. Stationary tube cultures incubated at 35 C at slightly inclined position were employed The medium was Parker 199 with Hank solution containing 1 g sod um bicarbonate per litre and su pplemented w th antibiotics and with 10 inactivated calf serum for out growth of cells and % of the same serum for maintenance of inoculated cultures. It was changed every 3 days.

### Virus

The RK 13-adapted Judith strain of rubella virus was supplied by Dr K. McCarthy In our hands this virus strain causes pronounced and easily recognized cytopathic changes in stationary RK 13 cultures [17]. Seed viru consisted f fluid harvested at peak irus titer one day after a change of medium. After larification by low speed centrifugation it was mixed with the same volume of glycerol and stored in screw-capped t ibes t −50 C Individual vial were thawed for each experiment Infectivit titres of the same virus bat h, run as con trol in the neutralization tests, did not change significantly over a period of 11 months which indicates a high degree of tal l ty of rubella virus under these cond tions A separat periment indicated, that the stabilit of rubella virus was not depen dent on the adm ture of gl cerol, reported by Wulff et al. [19] to st bilize respiratory syncy tial virus.

### Neutral zat on tests

Undiluted non heated human serum was mixed w th the same volume f virus diluted to contain about 100 $TCD_{50}$ f J dith per noculum dose (0 1 ml). Nonheated rabbit serum, stored t 70 C and diluted 1/ w th phosphat buffered saline served as virus d luent

The serum virus mixture was incubated at 37°C for one hour and at +4°C overnight before inoculation into tubes w th RK 13 cells. Cult ures were inspected for cytopathic changes after 11 days at 35 C All positive sera neutralized the CPE in both t bes. A simultaneous control of serum w thout virus did never show any CPE, nor any inhibition f vaccinia irus in the exclusion test.

A number of positive sera were t trated by testing serial twofold lilutions of serum (1/1 – 1/128) as lescribed abov T tres are given as the reciprocal f the highest initial dilution of serum inhibiting the CPE in t least one of the two cultures inoculated

As a control and an alternative to the microscopic inspection for CPE in the titrations an exclusion technique with vaccinia

virus was developed which allows a convenient macroscopic observation of vaccinia plaques in cultures showing no rubella virus CPE. After the 11-day reading for rubella virus CPE the cultures were freed from excess CO by aeration and then inoculated with about 1000 $TCD_{50}$ of vaccinia virus (as titrated in RK 13-cultures) Four or five days later the cultures were inspected macroscopically for focal degeneration. End point titres of rubella virus or antibody were almost identical with both types of readings since only an occasional end point culture negative t the 11-day inspection showed rubella type instead of vaccinial degeneration 4–5 days later

## Epidemiological Background

By courtesy of the Medical Board of the Armed Forces, statistics on rubella in conscripts of the Army during 1920–1949 and of the Armed Forces during 1950–1965 were made available. They showed a recurrence of epidemics approximately every five years as indicated by maxima in 1920, 1924–25, 1929–30, 1936–37 1941 1950–51 and 1955–56. A high incidence was noted in the consecutive years 1960–64.

Histories of previous attacks of exanthematic diseases were obtained for all persons who provided blood specimens.

## Results

The distribution of individuals with and without rubella antibody is shown in Table 1 and Fig 1 and 2. Cord bloods were positive in 83 %. After the disappearence of maternal antibody the frequency of subjects with antibody increased by advancing age (Fig 1). The children in the 2 year group were all susceptible the 5-year group was seropositive in 14 %, the 10-year-group in 51 %. The younger women, 15 20, and 25 years of age taken together had antibody in 73 %, whereas women 30 years and over were seropositive in 96 %.

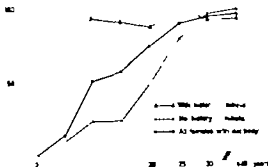


Fig 1 Frequency of females in Eskilstuna, Sweden, with antibody against rubella virus. Each age group comprises 35 individuals.

The proportion of women with past histories of rubella was highest in the 20-year group, 66 %, lower among both the younger and the older individuals.

A comparison of the frequency of antibody in females with a history of rubella with that of females without knowledge of a previous attack of the disease showed a significant difference 91 % of those with a history of rubella being seropositive compared to 4 % seropositive subjects in those without a history This was most marked

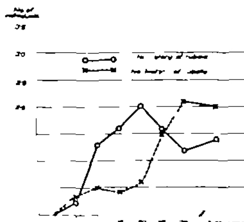


Fig 2 Number of females with antibody against rubella virus as related to history of rubella. Each age group comprises 35 individuals.

TABLE 1. *Rubella antibody in females in relation to reported experience of overt rubella*

| Group | Number tested | With history of rubella: With antibody | With history of rubella: Without antibody | With history of rubella: Total | With history of rubella: % Pos. | No history of rubella: With antibody | No history of rubella: Without antibody | No history of rubella: Total | No history of rubella: % Pos. | Total: With antibody | Total: Without antibody | Total: % Pos. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Newborn | 33 | 17 | | 18 | 89 | 1 | 4 | 16 | 75 | 29 | 6 | 83 |
| ? years (born 1963) | 33 | — | — | — | — | 0 | 33 | 0 | 0 | 0 | 33 | 0 |
| 5 years (born 196?) | 33 | 2 | 1 | 3 | 67 | 3 | ?9 | 32 | 9 | 5 | 30 | 14 |
| 10 years (born 1955) | 33 | 13 | 1 | 14 | 93 | 5 | 16 | 1 | 4 | 18 | 17 | 51 |
| 15 years (born 1950) | 33 | 16 | | 18 | 80 | 4 | 13 | 1 | 4 | ?0 | 15 | 5? |
| ?0 years (born 1945) | 33 | ?0 | 3 | ?3 | 87 | 8 | 6 | 1 | ?0 | ?8 | 9 | 74 |
| ?5 years (born 1940) | 33 | 16 | — | 16 | 100 | 15 | 4 | 19 | 79 | 31 | 4 | 89 |
| 30 years (born 1935) | 33 | 1 | 1 | 13 | 9 | 1 | 1 | ?? | 95 | 33 | | 94 |
| 40 years (born 1925 or earlier) | 33 | 14 | 1 | 15 | 93 | ?0 | — | ?0 | 100 | 34 | 1 | 9? |
| Total ≥ 15 years | 175 | 78 | 7 | 85 | 9?? | 66 | 4 | 90 | ?3 | 144 | 31 | 82 |
| Total – 5 and 10 years | 103 | 15 | 2 | 17 | 88 | 8 | 80 | 88 | 9 | 23 | 8 | 22 |
| Total ≥ 40 years | ?80 | 93 | 9 | 10? | 91 | 74 | 104 | 178 | 42 | 167 | 113 | 60 |

95 % confidence intervals: 84 97 %, 64 99 %, 8 -81 [illegible], 4 1 [illegible] %.

For newborn the figures denote if mothers have had rubella or not.

in the youngest girls, 5 and 10 years of age, 88 % being seropositive among those with history of rubella compared to only 9 % among those who had no history of rubella. The difference was significant also for the total of individuals 15 years and more, 9? % being seropositive among those with a rubella history compared to 73 % among those without. Of women 30 years and more the percentage of seropositives was similar whether or not a history of rubella was reported (98 and 93 % respectively).

Fig. ? shows the distribution of seropositive individuals according to history of rubella. In the age groups ?0 years and less 51 out of 69 seropositive females (74 %) had a history of rubella whereas the corresponding figures were 4? out of 98 (43 %) for the older age groups. The difference is significant ($p < 0.001$).

TABLE 2. *Distribution of titre of rubella NT antibody according to age*

| Titre | Age in years: 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|
| 1 | | | | | | 1 |
| 4 | | | | | 1 | |
| 8 | 2 | 1 | 1 | | 1 | 1 |
| 16 | | 1 | 5 | 1 | 3 | 6 |
| 32 | | 4 | 3 | 1 | | 4 |
| 64 | 1 | 4 | 7 | 1 | | 2 |
| ≥128 | | 6 | 3 | 1 | | 1 |

The neutralization titre was determined in 64 of the 107 positive sera (cord sera exempted). As seen in Table 2, titre values were rather scattered ranging between 1 and ≥128. There was a greater incidence of high titres in the lower age groups of 5–10 and 15–20 years compared to that of 25–30 years ($p < 0.01$ and $p < 0.05$, res-

TABLE 3 *Distribution of titres of rubella NT antibody according to history of rubella*

| Titre | With history of rubella | \ history of rubella | Rubella < 6 years ago | Rubella ≥ 9 years ago |
|---|---|---|---|---|
| 1 | | 1 | | |
| 2 | | | | |
| 4 | | 1 | | |
| 8 | 2 | 4 | 1 | 1 |
| 16 | 6 | 10 | 3 | 3 |
| 32 | 8 | 4 | 3 | 3 |
| 64 | 14 | 1 | 11 | 3 |
| > 128 | 9 | 4 | 8 | 0 |

pectively as calculated by Wilcoxon s Rank-sum test) Similarly a greater incl dence of high titres was found in persons with a more recent rubella infection ($p < 0.01$ for infections ≤6 years compared to those ≥9 years ago) and also in subjects with a history of rubella compared to those without ($p < 0.01$) (Table 3).

There was no correlation between ex perience of measles, scarlet fever or vari cellae and presence of antibody to rubella

## Discussion

The finding in this sample of the Swedish female population that immunity to rubella as determined by serology in creased by advancing age was to be ex pected and corresponds to the reports from Canada and U.S.A. mentioned above The rise in the frequency of girls with antibody from 14 in the 5-year-group to 51 % in the 10-year-group may reflect increased exposure to rubella after school entrance which in Sweden mostly occurs at the ge of seven, in connection with the relatively high incidence of rubella in recent years as revealed by the statistics carried by the Armed Forces This is the only reliable source of epidemiological information since rubella is not a reportable disease in Sweden. Previous experience indicates, how ever that rubella epidemics in military personnel are correlated to a high incidence of the disease among civilians.

The finding of serological immunity in 82 % of females 15 years and over is well in accordance with previous findings in North America and England as summarized above In the present series there was a demonstrable association of seroimmunity and history of rubella, not found in previous studies. Although this association was most evident in the younger age groups, it was significant also for women 15 years of age and above. The frequency of antibody in women 15 years and over with histories of rubella was 92 %, indicating a more widespread immunity in those with histories of rubella than in the women of corresponding age with no histories who showed antibody in a frequency of 73 %.

Our findings indicate that overt illness dominated among the persons up to 20 years of age (Fig 2) The relative increase of seropositive individuals without a history of rubella in the older groups, on the other hand, seems reasonable as an ttack of rubella in childhood should be more difficult to recall by advancing age It seems probable furthermore that reports are less reliable for women who suffered overt illness before the teratogenic effect of rubella infection was more widely realized in Sweden in connection with the wide spread epidemic in 1951 [ ] In fact only a few reports of rubella in childhood were given by w men more than 0 years of age

Other factors lthough less probable

may have contributed to lack of rubella history in a high proportion of older seropositive women, such as a higher rate of inapparent infections in earlier epidemics and/or in adult

Antibody titres obviously tended to *drop with increasing time after infection*. The higher incidence of low values in persons lacking history of rubella was probably also due to the ignorance of childhood rubella in the older age groups.

The distribution of titre values suggests that a drop in titre below the level recognizable by the technique used is uncommon, at least during childbearing age. The hypothetical problem of reinfections, discussed by Givan *et al.* [4] should therefore not be over-estimated. On the other hand, about 10 % of our subjects with a history of rubella did lack antibody and this incidence has been reported to be somewhat higher by Sever *et al.* [17]. It seems likely, however, that such a history in seronegative individuals is largely referable to other exanthematic diseases for example echovirus infections, measles infection, mononucleosis, scarlet fever and exanthema subitum. Thus a history of rubella does not guarantee immunity.

The pilot studies, so far carried out in U.S.A., Canada, Great Britain and Sweden, although by different laboratory techniques, suggest that about 1/5 of women of childbearing age are susceptible. In these countries, the incidence being higher in Hawaii and Japan. The need for prophylaxis against maternal rubella is thus documented.

## Summary

A total of 316 serum specimens obtained in Eskilstuna, Sweden, from females of different age groups were tested for neutralizing antibody to rubella virus. Lack of antibody in undiluted serum suggested susceptibility to rubella in approximately 1/5 of women at childbearing age. There was a significant correlation between reported history of overt rubella and presence of antibody. 91 % of all individuals 9 years and more with history of rubella being seropositive compared to 4 % among those without a history of rubella. In children 2.5 and 10 years of age with history of rubella 88 % were seropositive compared to only 0% in those without history. Serological immunity in the age groups 15 years and more was present in 9 % of the females with history compared to 73% of those without history of rubella, this difference also being significant. Women 30 years or more were seropositive in about 93 % regardless of history of rubella. Titre values were scattered with a tendency towards a greater incidence of high titres in the lower age groups and also in those with a history of rubella, particularly a recent one. Low titres in the women belonging to higher age groups and in those without history of rubella suggest that remote attacks were probably often forgotten.

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REVIEW ARTICLE

# Congenital Choanal Atresia

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Congenital choanal atresia (CCA) is a congenital closure between the nasal cavity and the pharynx consisting usually of a bony or bony membraneous plate extending from the horizontal plate of the palatine bone running obliquely upwards and backwards towards the body of the sphenoid bone and the ala of vomer. Medially it is continuous with the vomer and the nasal crest of the palatine bone, laterally with the internal pterygoid plate and the perpendicular plate of the palatine bone. The plate which is covered with a mucous membrane of the same structure as that covering the adjacent structures [8], is thinnest at its middle part, usually not more than 1 or 2 mm thick, but it may exceed 10 mm in thickness (Fig. 1). The atresia may be unilateral or bilateral.

The condition has been known definitely *for 150 years [22]. More than 100 years* ago the first successful bilateral surgical correction was undertaken by Emmert [9] in a 7 year-old boy.

A detailed description of the symptoms in bilateral CCA in a newborn infant was published by Ronaldson in *1880* [14].

In an exhaustive survey Durward *et al* [8] collected all published cases and found that up to 1954 not more than 500 cases had been reported, about two thirds of which were unilateral.

The actual incidence of CCA cannot be assessed on the basis of the available literature because presumably many cases, in particular bilateral cases, are not diagnosed and the causes of death in such infants are classified as congenital debility, respiratory distress, etc [3]. In most cases unilateral CCA is compatible with life and the symptoms are of such a constant nature that sooner or later the patient is obliged to seek medical advice. Johnsen's material from the Copenhagen University Hospital (Rigshospitalet) [13] comprising a total of 10 cases covering the years from 1953 to 1958 gives an impression of the incidence of the disease in the service area of the University Hospital.

The anomaly seems to be twice as fre*quent in girls as in boys [8], and to occur* twice as frequently in the right side as in the left [6, 10].

CCA is supposed to develop in several ways (Fig. 2 a–d). It is most probable that the formation of the bony atresia is caused by *failure of absorption* of the naso-buccal membrane which takes place normally during the 6th week. During the subsequent development the membrane




Fig. 1 Anatomical relationship of choanal atresia (A). (After McKibben [19]).

will be drawn backwards as growth of the palatine processes proceeds [1 5, 19]. The rare cases of membranous CCA may be caused by failure of absorption of the bucco-pharyngeal membrane [2 5]

Most cases of CCA occur sporadically although familial accumulation has been reported. As appears from T ble 1 CCA is frequently associated with other con genital malformations as e.g ventricular septal defect imperforat anus, and occa sionally with retrovaginal fistula This is explained by the fact that these structures undergo differentiation at the same stage as the choana [1 1] Furthermore it is stated that frequently CCA is associated with bifid uvula bilateral double tragu coloboma of the iris and retina [17].

CCA will not necessarily give rise to abnormal development of the nasal cavity or the sinuses, and the ciliary function of the mucous membrane is not ffected. Associated deformities of the middle ear nd facial asymmetry are rare [1 17].

## Symptomatology

*B'lateral CCA*

A newborn infant breathes through the nose only Up to the age of 5 months, infants will only breath through the mouth while crying After that age the mouth is opened voluntarily when the nose is occluded [3] In bilateral CCA, therefore, the newborn infant will present severe respiratory distress with retraction of the chest and occasionally of the cheeks, in dicating severe obstruction of the upper air passages. If the child starts crying, breathing will come freely the symptoms disappear the child becomes quiet, closes his mouth and the respiratory distress returns. This cyclic respiratory distress is characteristic of CCA [23 24]. If no treat ment is instituted, most infants will die from asphyxia although, as it appears from reports in the literature, a few may survive [4 5 6 9 23 24 25] During the breast-feeding period especially the patients will be very distressed while suck ling. In adult patients the most troublesome symptoms will be of nasal nature There will be constant nasal discharge, and the nose cannot be cleared by blowing, the so-called "silent collection of discharge On crying the nasal discharge becomes more pronounced The skin around the nares will be irritated nd macerated because of the constant discharge The facial expression will be f an adenoid character and the speech will be nasal. The mucous membrane in the mouth nd pharynx will be dry because of mouth breathing Conversely infections of pharynx, rhinopharynx th Eustachian tube or the tympanic cavity are not remarkably frequent

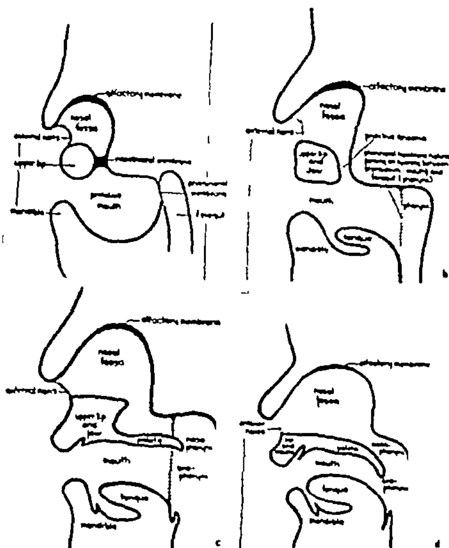


Fig. [illegible]–d. Development of [illegible] (after Colver [6]). a, 4th embryological week; b, 6th–7th embryological weeks. Bony type of choanal atresia probably caused by persistence of nasobuccal membrane. Membraneous choanal atresia may be caused by persistence of cranial part of pharyngeal membrane

*Unilateral CCA*

Local symptoms such as are seen in bilateral atresia will be predominant, but restricted to the affected side. However mouth breathing, stertorous breathing and nasal speech will often be present. Conversely, in infants respiratory distress appears only if the unaffected nostril is occluded by compression or by discharge.

## Complications

In some cases purulent discharge will develop because of rhinitis. Complicating sinuitis has been reported as the cause of

TABLE 1 *Congenital choanal atresia and conjoined malformations*

| Author | References | Type of atresia | Malformations |
|---|---|---|---|
| Colver | 5 | Bilateral | Mental retardation |
| Connelly | 6 | Unilateral | Mb cord. cong spina bifida cervicalis occulta, hypoplasia opposite side of body |
| | | Bilateral | Mb. cord. cong. |
| | | Unilateral | Fistula oesophago-tracheal., anus imperf., fistula recto-vaginal. |
| Craig | 7 | Unilateral | Mb. cord. cong |
| | | Bilateral | Malrotatio intest., divert. Meckelii |
| Fałeg | 11 | Unilateral | Microphtalmia, tumor dermoid anomalia facial. hypophys hypothalamoid disease |
| Flake & Ferguson | 12 | | Coloboma iridis |
| | | | Coloboma iridis |
| | | | Mb. cord. cong. |
| | | | Atresia oesophagi |
| | | | Fistula oesophago-trachealis |
| | | | Meningocele |
| | | | Synostosis cranii |
| Johnsen | 13 | Unilateral | Mb. cord. cong |
| | | Unilateral | Mb. cord. cong |
| McGovern | 16 | Bilateral | Mb. cord. cong. |
| | | Unilateral | Mb. cord. cong |
| | 17 | Bilateral | Syndaktylia |
| | | | Uvula bifida |
| | | | Coloboma iridis |
| | | | Tragus bifidus bilat. |
| | | | Fistula branchialis bilat. |
| | | | Polydaktylia |
| | 18 | Unilateral | Syndroma Turner |
| McNeall & Wynter Wedderburn | 20 | Bilateral | Syndroma Treacher-Collins |
| Hoboth, Buchmann & Sandberg | | Bilateral | Anus imperforatus, anus vestibulare |
| | | Bilateral | Hydrocephalus intern., mental retardation |
| | | Bilateral | Mb. cord. cong |
| | | Bilateral | Mb cord. cong. |

pyæmia and death, but this complication seems to be rare supposedly because the ciliary function of the epithelium is preserved [8, 18]. Otitis media does not seem to be more frequent in patients with CCA than in the general population.

## Diagnosis

In all newborn infants revealing respiratory distress, particularly when such distress is of a cyclic nature CCA should be suspected. The diagnosis is verified by demonstration of an occlusion of the passage between the nasal cavity and the rhinopharynx This is carried out most easily by probing with a Nelaton or Thiemann catheter No. 8 since normally in a newborn infant such catheters can be passed easily through the choanae down into the pharynx. Instillation of a dye or radiographic examination after installation of a radio-opaque substance can also be employed (Fig 3)

Unilateral CCA should be suspected in the presence of unilateral nasal discharge at birth The diagnosis is also based on



Fig. 3. Patient N. 3. Contrast medium injected in nasal cavity demonstrating choanal closure. Note: oro-gastric tube flowing at passage through the mouth.

failure in probing the choanal passage. In older patients it may be diagnosed by posterior rhinoscopy.

*Differential diagnoses:* Diseases simulating bilateral CCA are neonatal asphyxia, congenital debility, pulmonary atelectasis, respiratory distress, heart disease and cerebral haemorrhage. A faulty diagnosis of unilateral CCA may be made only in cases of severe deviated nasal septum or obstruction by a foreign body.

## Treatment

### *Medical treatment*

Bilateral CCA requires emergency treatment so as to preserve the life of the newborn infant. Unilateral cases do not require emergency measures but elective surgical correction at a later date.

Immediate steps must be taken to ensure passage of air through the mouth. This is effected most easily by means of a tongue depressor, e.g. Guekden's. If such a depressor is not available the mouth is kept open by means of a gastric tube, not too thin, which is passed through the mouth down into the stomach. For more prolonged treatment various devices have been used, e.g. a rubber nipple with holes 0.5 cm large [15], a plastic tongue depressor [10], an oral plate [14].

Conservative treatment requires careful supervision of the child, because dislocation of the tongue depressor may prove fatal. At the beginning feeding has to be by gastric tube.

### *Surgical treatment*

The ultimate aim of treatment is to create normal air passage through the nose. All symptoms are hereby relieved.

Resection of the obstruction by the transnasal approach is the simplest method of operation. It is preferred in newborn infants in spite of obvious technical disadvantages. For the purpose of maintaining patency of the passage plastic tubes are inserted into the choana and remain in place for the first 10 days after operation, thereafter daily bougie treatments are given during the first period. Notwithstanding these measures secondary closure may often occur, requiring re-operation. Beinfield's modified technique [2] aims at covering with mucous membrane, whereby the tendency to recurrence is reduced.

In older children and adults the transpalatinal operation is employed whereby a better view and a wider approach to the atresia are obtained. Several technical modifications of this method have been described, each with the object of removing as much of the atresia as possible, often together with the posterior part of the

TABLE 2. *Congenital choanal atresia. Therapy and results. Copenhagen County Hospital in Gentofte 1961–1960*

| Pa-tient No. | Sex | Oc-cluded side | Conjoint malfor-mation | Age at opera-tion | Opera-tion | Pri-mary result | Time be-tween first and second operation | Final result | Follow up period | Special remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♂ | Bilat | Mb. cord. cg. | 1 day | N[a] | Tempo-rary | 3 months | Unsa-tisf. | 6 months | Dead 6 months old from cong. heart disease |
|  | ♀ | Bilat. | Anus vestib. | days | N | Success-ful |  | Excel-lent | 4 years |  |
| 3 | ♂ | Bilat. | Hydroceph. | 2 days | N | Tempo-rary | 2 months | Good | 1 year | Reoperation twice |
| 4 | ♀ | Bilat. |  | 6 days | N | Success-ful |  | Good | 3 months | Left the country while still having dilatations of the choanae |
| 5 | ♂ | Bilat. | Mb. cord. cg |  |  |  |  |  |  | Conservativ treatment with oral device, aiming at transpal. operation 18 month old. |
| 6 | ♀ | Right | Uvula bif | 5 years | P[b] | Success-ful |  | Excel-lent | 2½ years |  |
| 7 | ♀ | Right | Uvula bif. | 16 years | P | Success-ful |  | Excel-lent | 3 years |  |
| 8 | ♀ | Right | Uvula bif. | 40 years | P | Tempo-rary | 4 months | Excel-lent | 4 years | Membranous occlusion after first operation. |

[a] Transnasal. [b] Transpalatinal.

vomer and the hard palate The endeavour of the operation is to leave the bony edges with a cover of mucous membrane if necessary by slight compression, g by the insertion of a plastic tube In general, the results of such operations are good. Because of the limited space this technique is not commonly used in newborn infants

## Own m terial

Since 1961 8 patients with CCA, 3 unilateral, right-sided, and 5 bilateral, have been admitted t the Copenhagen County Hospital in Gentofte.

All cases of bilateral CCA were admitted t the pediatric department with varying diagnoses shortly after birth. The final diag nosi was made by failure in passing cathe-ter through the choanae

The first four children admitted were operated on within the first six weeks of lif (Table *). Four of the hildren had other malformations, viz.: two with heart disease one with vestibular anus, and one with hydrocephalus. Three of the infants were fully grown, one weighed 2000 g and one 2400 g

All operations were performed under general naesthesia with oral intubation. No surgical mortality was noted. As ppears from Table 2, the primary result of the operations were good in ll the cases. In two of the infants, however the choanae closed again in spit of energetic bougie treatment and the operation had to be repeated. A second surgical correction had to be perfor med in child N 1 at the age of 3 months but in this case also, the operation produced only temporary relief However her general con dition did not permit further correction, and he died from congenital heart disease t the ge of 6 months. Child No. 3 underwent operation for the second and third time t the age of 2 months and 11 months, respectively the latter time after admission to another hospital The child has been followed up for



Fig. 4. First maxillary acrylic base plat for patient No. 5, seen from underneath. T the right extra oral funnel. Not the too-acute angle on nt be t the left

1 year and the res lt continues to be good. In ch ldren Nos and 4 good primary results were found. these children were followed up for 4 years and 3 months respectively.

One patient with bilateral CCA was treated without surgical correction. Patent air passage was ensured by means of maxillary acrylic base plate with built in air duct (Figs. 4 and 5)[1] Treatment was terminated at the age of 5 months.

In three patients, 5, 16, and 49 years of age respectively unilateral CCA was diagnosed. Surgical repair produced good primary result in the two younger patients, whereas in patient No. 8 a woman aged 49 a membranous occlusion developed after the first operation and consequently second correction had to be made 4 months later. The result was now complet ly satisfactory.



Fig. 5. X-ray lateral view of patient No. 5 with the first base plate in situ. Not base of tongue depressed and pushed forward by the plate leaving diastasis between plate and uvula.

[1] Constructed by P. Hildestad, Assistant Professor Prosthetic Department, Royal Dental College Copenhagen.

## Discu ion

Whereas unilateral CCA does not present special problems in respect of the survival of the individual, bilateral CCA may well prove fatal but can be corrected by relatively simple mea ures. Su picion of this disease is aroused by the characteristic respiratory distress in particular the peculiar effect of crying. The diagnosis is made by probing the nose. In all our cases rubber catheters were used, and there were no faulty diagnoses. Metal probes may give an impression of the nature of the atresia, but in other respects they do not offer diagnostic advantages. In a few patients the diagnosis was verified by radiographic examination after instillation of a contrast medium into the nasal cavity but in no case was this examination necessary in order to settle the diagnosis.

Cases of choanal stenosis have been encountered which were diagnosed as CCA by probing the nose and by examinations using a contrast medium. From a functional standpoint these cases must be regarded as atresias.

It is not possible to state any definite incidence of CCA. Presumably a number of cases will remain undiagnosed also at autopsy because examination for CCA is not carried out as routine at post mortem

studies. During 5 years we have encountered 5 cases of bilateral CCA from a service area with a birth rate of nearly 40,000 corresponding to an incidence of about 1 per 8000 births.

At the beginning the treatment in newborn patients consisted in the establishment of a passage through the nose as soon as possible. Four infants were operated on within the first week of life and in all these cases the transnasal approach was employed. It was a common feature of the post-operative course in the newborn infants, that this period was very trying for the patients as well as for the medical and nursing staff on account of the numerous irrigations and bougie treatments that were necessary A total of three reoperations were carried out in two patients.

One patient died from a complicating congenital heart defect. This case seems to be analogous to the case reported by McGovern [16] since our patient had a patent ductus arteriosus and right ventricular hypertrophy

The results of surgical correction in our first four patients with bilateral CCA show that the transnasal approach is not an ideal method. Conversely the outcome of the transpalatinal approaches in unilateral CCA shows that this surgical technique may produce a lasting and favourable result As other surgeons have reported the same experience and since it has been shown that it is possible to postpone operation of a bilateral CCA until the child is old enough to undergo transpalatinal operation [10, 14 16], we decided on conservative treatment in our patient No 5. The oral plate was constructed in the conventional manner by taking a cast of the palate and the gingivae It is of importance that the posterior part of the plate is curved sufficiently without forcing the soft palate or the uvula backwards towards the posterior wall of the pharynx, but nevertheless keeping the outlet of the respiratory passage free of the root of the tongue A radiograph in the lateral projection with the plate in position is an important check (Fig 5) The plate is fixed with Tragacant (Dentofix[1]) and must be changed and cleaned after each meal. It was necessary to renew the plate approximately each fourth week because of the child's growth.

For the first few days the patient was fed through a tube but soon he learnt to drink without the plate in his mouth, but not without difficulty The child was discharged at the age of 3 months On a follow-up examination at the age of 5 months the child fared well and the plate was no longer necessary

The question as to the time at which these patients should undergo operation, remains to be solved. At the age of 18 months the space is twice as large as at birth, and the child has not yet started speaking In our opinion this age is the optimum for surgical correction.

## Summary

Unilateral congenital choanal atresia (CCA) may in all essential present local symptoms and does not require emergency measures

Bilateral CCA becomes manifest immediately fter birth and presents a characteristic picture cyclic dyspnoea and cyanosis, which disappear on crying discharge from the nares and difficulty in

feeding. If treatment is not instituted the condition may prove fatal.

From a technical point of view emergency surgical correction i extremely difficult on newborn infant and a considerable frequency of recurrence i seen. Therefore we prefer immediate conservative therapy with an oral plate whereby free mouth breathing is ensured. Surgical repair can be carried out by employing a better and safer technique when the child i about 18 months old.

Three right-sided and five bilateral cases of CCA are presented. Four patients with bilateral CCA were operated on during the neonatal period one was treated with a maxillary acrylic base plate to ensure a patent air passage.

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CASE REPORT

# Familial Progressive Poliodystrophy with Cirrhosis of the Liver

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The purpose of this report is to describe the clinical and pathological findings in two siblings who died after a very similar clinical course characterized by convulsions, mental and statomotoric retardation and jaundice in the last weeks of the disease. The main postmortem findings were atrophy of the cerebral gray matter and cirrhosis of the liver.

## Case report

There is no information on hereditary disorders in the family; no known consanguinity between the parents. They have had 7 children, 4 are alive and healthy, 1 died accidentally. Child no. 2 and 5 suffered from the disorder to be described.

The first patient was a girl, born in 1955, the second a boy born in 1962. Due to the close similarity between the two patients, only the second one will be described in detail. At the time of the second patient's birth his mother was 31 and his father 35 years old. The pregnancy, delivery and neonatal period were uncomplicated. The birth weight was 4400 g. His motoric development was slightly retarded. He was able to sit with support when 5–6 months old, without support at 10 months of age.

At 14 months he was admitted to hospital in status epilepticus. The convulsions, which were localized to the left side, lasted for more than 24 hours despite treatment with phenobarbital and chloral hydrate. Finally ether narcosis stopped the convulsions. The patient was febrile. The spinal fluid was normal. No retinal abnormality was observed. Electro-encephalography showed focal theta-delta activity and reduced, fast activity in the posterior part of the right hemisphere. There was a normal, low excretion of amino acids in the urine. Gradually the patient awoke and showed contact with his surroundings. Following the convulsive state he had a left side hemiparesis which lasted for 17 days.

When 16 months old he again had a long convulsive attack accompanied by fever. His condition improved after a few days. There was no apparent impairment of hearing or vision, but he showed signs of motorical and mental retardation.

At the age of 18 months he was again admitted in status epilepticus; this time with convulsions mainly on the right side. Intensive antiepileptic medication had some effect and he awoke, but for the following two weeks he had more and less continuous fits, reminiscent of epilepsia partialis continua.

Following vomiting and aspiration to the bronchial tree he developed pneumonia. Gradually he became more limp and drowsy with hypotonic muscles. In the last month of life he had no convulsions and the antiepileptic drugs were reduced in dosage. 2

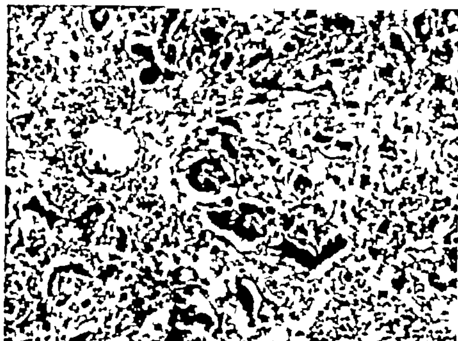


Fig. 1. Case 2. Microphotograph of the liver with depletion of liver cells and proliferation of connective tissue with bile ducts and leukocyte infiltration. Haematoxylin and eosine × 110.

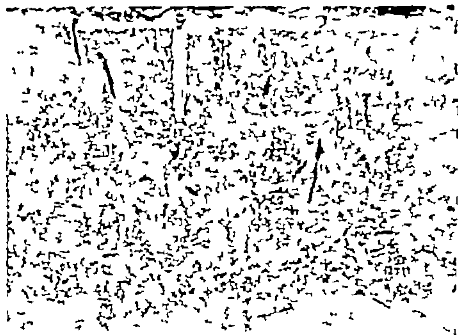


Fig. 2. Case 2. Microphotograph of occipital cortex showing laminar spongy degeneration. Haematoxylin and eosine × 80.

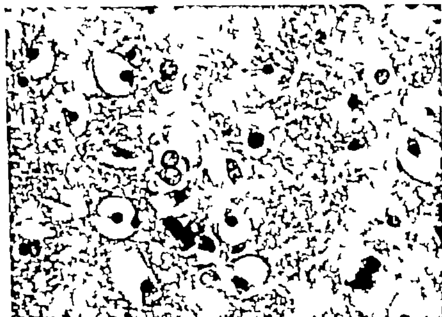


Fig. 3. Case 2. Nerve cell depletion and proliferation of glia cells with large, pale nuclei in parietal cortex. Haematoxylin and eosine × 600.

weeks prior to death jaundice was noticed. The serum transaminase was elevated. Prothrombin was low despite administration of vitamin K. A septic state with jaundice was suspected and he was given antibiotics, without effect on his clinical condition. He died at the age of 20 months.

## Autopsy findings

At postmortem an atrophic bile stained liver was found in both siblings. The extrahepatic bile ducts were normal. Microscopically the liver showed marked degeneration and cell depletion. The liver parenchyma was replaced by loose proliferating connective tissue with multiple bile ducts and leucocyte infiltration (Fig. 1). In the first case the pancreas showed degenerative changes with leucocyte infiltration and fat necrosis. In the second case the pancreas showed slight interstitial fibrosis, but no inflammation. There were no signs of mucoviscidosis.

In both siblings the brain was slightly reduced in size with gross and microscopic atrophy of the cerebral cortex. No sign of dysplasia was found. In all areas but most marked in the parieto-occipital regions, the cortex showed a laminar spongy degeneration, especially in the 2nd–3rd and in the 5th cortical layers (Fig. 2). There was degeneration and depletion of nerve cell and proliferation of astroglia. The glial proliferation was most marked in the deeper layers of the cortex, where the glia cells showed large pale, ballooned nuclei and scanty cytoplasm (Fig. 3). In the subcortical white matter similar changes of the glia cells were found in lesser degree. There was no spongy degeneration or signs of demyelination of the white matter. In the caudate nucleus and putamen, and to a lesser extent also in the thalamus and cerebellum, nerve cell degeneration and glia proliferation were noticed. There was no significant increase in free lipoid material. No metachromatic substance was noticed.

### Resumé

Two siblings developed convulsions at 11 and 14 months of age and subsequently became limp and hypotonic with dementia. Four and two weeks prior to death they became jaundiced. At postmortem examination laminar spongy degeneration of the cerebral cortex was found with many large swollen glia cells. The liver showed subtotal atrophy with fibrosis, inflammation and bile duct proliferation.


### Discussion

Heredo-degenerative disorders of the central nervous system are difficult to classify clinically. The etiology and pathogenesis of most types are unknown. Different etiological agents may presumably cause similar morphological changes and clinical symptoms due to the limited reactive possibilities of the cerebral tissue against damaging agents. Classification is made according to the pathological findings as seen at autopsy.

The central nervous system in our two patients showed pathological changes mainly in the gray matter. The cortical lesions may be called poliodystrophy and are comparable to poliodystrophia progressiva as described by Alpers [ ] and later by Christensen & Krabbe [4]. The etiology of poliodystrophy is unknown, but some factors have been thought to be of etiological or pathogenetic importance. The following classification has been proposed [6, 7, 11]: 1) Familial type, 2) Post traumatic, 3) Postepileptic, 4) Postinflammatory and 5) Subacute infantile cortical necrosis. Probably cerebral poliodystrophy is no etiological entity, but a pathological lesion which may be caused by different noxious agents. The etiology of the lesions in our cases is unexplained, but it is probable that longlasting convulsions may have been of pathogenetic importance for the observed laminar cortical atrophy with degenerative changes in basal ganglia and cerebellum.

On clinical grounds two types of poliodystrophy have been described [6]. In the juvenile type the clinical symptoms start at 3–6 years of age. There is a slow progression until death some years later. The infantile type starts usually before one year of age and ends fatally before 4 years of age. In the infantile type convulsions are the predominating sign. The convulsions are quite often therapy resistant and are followed by pareses. Myoclonic seizures are described in some cases, as are muscular hypotonia and rigidity. The disease has no characteristic symptom apart from convulsions and progressive dementia. The diagnosis is probably impossible without biopsy or autopsy.

In the last stage of the disease our patients showed signs of liver failure. Marked pathological changes in the liver were found at postmortem, indicating that the liver disease had been of longer duration than the period of jaundice. Both hereditary and acquired liver diseases with liver failure may give cerebral damage with neurological symptoms. In our cases the large number of large, swollen glia cells found at postmortem may be secondary to the terminal liver failure. The cerebral symptoms, however, predominated throughout the first part of the disease, making it difficult to assume that they were entirely due to liver failure.

Both patients were given large doses of antiepileptic drugs. Liver damage has been noticed following administration of phenobarbital and phenytoin. Both infants were tube fed periodically. The liver damage might possibly be secondary to a protein insufficient diet in combination with antiepileptic drugs. This explanation, however, does not seem likely. A more probable explanation seems to be the existence of a familial metabolic defect causing the cerebral damage as well as the liver failure.

Combined hepato-cerebral degeneration is the characteristic feature of Wilson's disease. However, the earliest age of onset in Wilson's disease is recorded as 4 years according to Cumings (6). Furthermore the clinical course was unusual and the copper content in liver tissue was normal. Tyrosinosis (8) and galactosaemia appear to be excluded and there was no evidence of other types of ammoaciduria. Encephalopathy with visceral fatty degeneration due t[illegible] xogenic factors (0 1[illegible] 13) has been reported, but the histological pictur[illegible] differs from that observed in our cases. The interval of 7 years between the two patients renders an exogenic cause unlikely.

Recently Adams (1) reported two cases of portocaval shunt without signs of liver damage but with cerebral symptoms follo[illegible]ng the shunt operations. At post mortem laminar cortical degeneration with proliferation and enlargement of astroglia was found. The changes appear to be similar to those observed in our patient. If metabolic product normally present in the portal circulation can give brain damage it seems likely that abnormal product present in the general circulation due to metabolic errors could give cerebral damage as well as hepatic degeneration. One other possible mechanism is a deactivated enzyme-system, a postulated pathogenesis of encephalopathy combined with deficiency of liver phosphorylase (10). In our first case high values for total serum lipids and phospholipids were found (6*00 mg/100 ml and 608 mg/100 ml, respectively). However these abnormally high values were obtained at a late stage after the onset of liver failure which might have been the cause of the hyperlipaemia. In the second case liver tissue analyzed gaschromatographically showed no abnormal fatty acids.

Blackwood and collaborators (3) in 1962 described 6 cases of poliodystrophy. In three cases a postmortem examination was performed. Two of them also siblings showed l[illegible]ver changes sim[illegible]lar to those found in our cases. The authors do not discuss the significance of the combined cerebral and hepatic degeneration. The report indicates that cerebral poliodystrophy may not infrequentl[illegible] be accompanied by liver damag[illegible]

Despit[illegible] the lack of evidence on etiological agents we believ[illegible] that we are dealing with a disease entity because of the familial occurrence and th[illegible] closely similar clinical signs and postmortem pathological changes observed in our cases.

## Summary

Two siblings both having shown slight statomotoric retardation, developed convulsions when 11 and 14 month old, respectively. Subsequently they had sev

eral convulsions and gradually became hypotonic with dementia. They developed jaundice 4 and 2 weeks before death at the age of 15 and 20 months respectively.

At postmortem examination laminar spongy degeneration of the cerebral cortex was found with depletion of nerve cells and many large swollen glia cells. The liver showed subtotal atrophy with fibrosis, inflammation and bile duct proliferation.

Cerebral poliodystrophy and liver cirrhosis in the same patient have been reported by Blackwood *et al.* [3]. The findings, however, were not considered etiologically related. Due to the close similarities of clinical course and pathological lesions shown by our cases, we believe that we are dealing with a disease entity. It is assumed that a congenital, metabolic defect may be present, but our cases permit no conclusions regarding the etiology.

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CASE REPORT

# Down's Syndrome and Transient Leukaemia like Disease in a Newborn

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A newborn full term female infant showed a habitus typical of Down's syndrome Chromosome studies gave the result 47/21 trisomy

On the basis of blood and bone marrow findings a diagnosis of acute leukaemia was made without reservation.

Without treatment the blood and bone marrow soon became normal and remained so at repeated check ups At th time of writing she is 4 years old.

## Course of the Disease

Sirpa S., born on September 8 1962. Birth weight 3300 g; 1/I-child. The father was healthy aged 20; the mother was healthy aged 24 t the time of delivery During pregnancy the mother was healthy except for three weeks before delivery when she had low back pain for which she was given Miltyl tablets (meprobamat 0... g, acetylsalicylic acid 0.33 g) Of these she took a maximum of ten. The urine was not examined. N roentgenologic examination was performed. Her blood group was B Rh+ The Wasserman reaction was negative. She worked until three weeks before delivery

The infant was admitted on September 10 1962, on account of peripheral cyanosis, difficulty in feeding and dyspnoea. The general condition was fairly good. A systolic murmur of degree III–IV was heard. The respiratory sounds were somewhat weaker over the right lung. The liver was palpated bout 2 cm and the spleen 1–1.5 cm, below the costal arch. One week later the liver extended as far as the umbilicus and the spleen was palpated 3 cm below the costal arch. The urine was normal. Bacterial cultures from the nose, throat and rectum showed a normal flora, in addition the rectal specimen showed growth of Monilia.

The blood group was B Rh+ Direct Coombs test was negative Serum bilirubin. direct 1 46, total 11 1 mg/100 ml. The leukocyt count was greatly elevated. Most of the cells were blast cells. (Table 1 Fig 1) The thrombotest was 40 %, the bleeding time 3'35 the coagulation time 35 1 3 In the bone marrow smears occasional normal cells from the myeloid and erythroid series were found but the majority of the cells were typical blast cells. Scanty fine granulation was seen in some cells, which obviously belonged to the myeloic series

Roentgenologic examination of the long bones, performed on September 28, demonstrated loss of calcium in the metaphyses and the cortex of the pelvic bones. A chest roentgenogram showed large heart which occupied a considerable part of the left half of the thorax.

Stated in brief the following features were noteworthy when the infant came under our treatment 1) Down syndrome 2) con

TABLE 1 *Haematological [illegible]*

| Date | | Haemoglobin (g/100 ml) | Erythrocytes (per mm³) | Reticulocytes (per cent) | Leucocytes (per mm³) | Stab neutrophils (per cent) | Segmented neutrophils (per cent) | Eosinophils (per cent) | Monocytes (per cent) | Lymphocytes (per cent) | Blast cells (per cent) | Platelets (per mm³) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1962 | | | | | | | | | | | | |
| Sept | 1 | 20.4 | 5.33 | | 97,000 | | 8 | 1.0 | | | 91 | |
| | | | | | 124,000 | 1.5 | 8 | | | | 90.5 | |
| | 4 | 20.4 | 5.02 | 0.8 | 100,000 | 1.5 | 6 | 0.5 | | …3 | 87.5 | 80,000 |
| | [illegible] | 19.5 | [illegible] | | 6,000 | | 11 | | | 24 | 65 | 78,000 |
| Oct | 30 | 13.5 | | 0.9 | 13,0… | 5 | 10.5 | | 5 | 77.5 | 2 | 161,000 |
| 1963 | | | | | | | | Basophil (per cent) | | | | |
| Dec | 5 | 13.8 | 3.60 | | 4,400 | | 33 | 2 | 3 | 61 | | 520,000 |

genital heart disease and slight cardiac insufficiency, 3) hepato- and splenomegaly, 4) leukocytosis, 5) granulocytopenia, 6) thrombocytopenia, 7) the majority of the blood and bone marrow cells were blast cells, 8) no anaemia was present, the reticulocyte count was low, 9) no signs of severe infection were observed, 10) changes of the bones of the type encountered in acute leukaemia were present.

The diagnosis of acute leukaemia in a newborn mongoloid infant with congenital heart disease was regarded as certain.

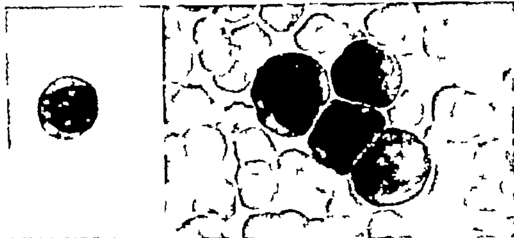
On September 20 digitalis treatment and prophylactic penicillin-streptomycin treatment was instituted. The liver gradually decreased in size and when the infant was 6 weeks old digitalis could be withdrawn.

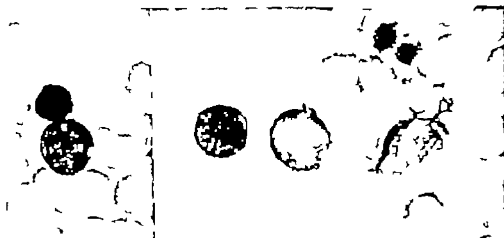
No therapeutic measures were taken on account of the leukaemia observed. Before long it was noted that the blood picture began to normalize. The leukocyte count gradually decreased although the percentage of blast cells remained high to begin with (65–78 %) (Table 1). In bone marrow specimens obtained on October 10 and November 6, 1962, erythropoiesis was normal, morphologically normal cells belonging to the myeloic series were abundant and the relation between the cells in different stages of maturation was normal. The smears contained 10 per cent and 5 per cent blast cells respectively. On October 31 1962, serum immunoelectrophoresis demonstrated complete absence of the γM-band, while the γA band was within the normal range. The γG band was very weak and indistinct.

## Follow-up Period

The patient was seen when she was aged one year and 3 months, one year and 11 months, 3 years and 4 months and 4 years (on September 16 1966). During the first year of life she had several respiratory infections, which were not severe. In December 1963 the liver was palpated about 1 cm below the costal arch. In spite of her heart disease the general condition remained good. The bone marrow and blood were normal (Table 1). In August 1964 in January and September 1966 she was well and the blood and bone marrow were still normal. In January 1966 serum immunoelectrophoresis showed an increased amount of γG-globulin. An examination for Mycoplasma antibodies using the indirect haemagglutination test gave a serum antibody titre of 1024 against

Fig. 1 Smears of the peripheral blood of the infant at 14 days of age (May-Grunwald Giemsa)







*Mycoplasma hominis* of type (Campo); in May 1966 the titre was 512 and in September 1966 the titre was 16. The serum immunoelectrophoresis gave a normal result. The anti-thyroglobulin titre (haemagglutination) was 25. The Sia test was strongly positive.

During the follow-up period a maternal uncle of the child died in 1963 and another maternal uncle died in 1965, both at the age of 34. They were not mongoloids. In both cases the cause of death was a myeloproliferative disease of some months' duration. In both cases the disease was malignant in nature and in both cases the bone marrow exhibited atypical cells which were difficult to identify. In both cases features occurred in the final stage, however, on account of which the consulting haematologists independently of each other made a diagnosis of subacute myeloid leukaemia. The mother, the maternal aunt and the surviving maternal uncle of the present child have all been examined for leukaemia and found to be healthy.

## *Discussion*

Acute leukaemia of the newborn is not uncommon. The question of congenital leukaemia and leukaemia-like conditions of the newborn has recently been discussed [1]. We have previously reported 4 cases of the disease [4, 1?]. In the present case the haematological picture with its very high leukocyte count, with atypical blast cells constituting the vast majority of the blood and bone marrow cells, was typical of leukaemia of the newborn. The liver and spleen were enlarged, and the bone changes observed were in agreement with this diagnosis. Furthermore, the patient exhibited typical Down's syndrome. In children with this syndrome the frequency of leukaemia is increased; it can be even 21 times higher than in the general population [10]. The incidence of leukaemia in newborn mongoloid infants is considerably higher than the incidence of the disease in the total age group 0–4 years [5, 8]. We have previously encountered such a case. The child had transposition of the great vessels and died at the age of 6 weeks.

The thymus in children with Down's syndrome frequently shows large cystic Hassal's bodies [2, 7]. It has been suggested that Hassal's bodies eliminate forbidden clones [6]. A reduced capacity of Hassal's bodies to eliminate leukaemic clones might be one reason for the increased rate of leukaemia in children with Down's syndrome [7]. The present child did not show any significant symptoms of impaired thymic function. During the follow-up period she had no severe infections and she had not lymphocytopenia. She had, however, thyroid antibodies and the Sia test was strongly positive. The result of serum immunoelectrophoresis when the child was 8 weeks of age showed the pattern of the mongoloid. When it was repeated when the child was 3 years 4 months and 4 years it was normal for the age.

Newborn infants suffering from leukaemia usually are seriously ill. A haemorrhagic diathesis, skin infiltrations and jaundice are often present. In the present case the general condition was fairly good and the patient exhibited none of the above-mentioned signs. The enlarged liver was in part, at least, due to cardiac insufficiency. On the other hand, the spleen was also enlarged. The features of the disease were not indicative of isoimmunization or of a severe acute or intrauterine infection.

The course of the disease argues against the diagnosis of congenital acute leukaemia, which as a rule has a violent course,

reacts poorly to treatment and relatively soon causes the death of the patient irrespective of treatment.

In acute leukaemia spontaneous remissions are known to occur but these are usually of short duration. The greatest incidence is found in young children [5]. Treatment may produce remissions which last for years.

In 1954 Schunk & Lehman [11] described a newborn mongoloid infant, which presented the clinical and haematological picture of acute leukaemia. The signs of leukaemia disappeared, and when the child died at the age of 6 years, no signs of this disease were detected.

In 1963 Ross *et al.* [9] described a newborn mongoloid infant, exhibiting a condition which resembled leukaemia. The haematological picture differed from that observed in the present case Initially the infant had 25 000 white blood cells, 4.. % of which were neutrophils and 54 % lymphocytes. When the infant was 14 days old, the leukocyte count w s 37 400 with 43 5 % myeloblasts and promyeloblasts. Therapy was instituted, and the blood and bone marrow were normalized. The authors concluded that the condition involved was ineffective granulopoiesis masquerading as leukaemia"

In 1964 Engel *et l.* [3] described 3 newborn mongoloid infants showing features typical of acute leukaemia In one case remission occurred when the infant was 2–3 months old, and at the age of 3 years the patient s bone marrow was still normal When the child died at the age of 3 years and 4 months no signs of cute leukaemia were observed. Another child showed remission at the age of 3 months and was still healthy at the age of 2 years and 10 months. The third child showed remission at the age of 3–4 months and was healthy when seen at the age of 9 months. None of these children received specific antileukaemic therapy

The conclusion is drawn that newborn mongoloid infants may exhibit features which are interpreted as acute leukaemia and which may normalize sometimes spontaneously as in the present case


## Summary

A newborn mongoloid female infant showed atypical blast cell infiltration in the bone marrow Ninety-one per cent of the same cells were observed in the blood, the total count being 130 000/mm³ when the infant was 14 days old. The blood and bone marrow normalized within about 6 weeks without any specific antileukaemic therapy At the age of 4 years the child was still healthy and the blood and bone marrow were normal Two maternal uncles of the child died as adults of a malignant myeloprolif rative disease


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## Addendum

Since our man script was prepared we have recei ed information (dr U Furuhjelm) that an apparently similar case a our patient recently ha been observed in the same hospital

A female newborn with Down syndrome and 1 trisomy w admitted there at the age of weeks. Peripheral blood and bone marrow were compatible with acute leukaemia of stem cell type No specific treatment was instituted and the haematological findings were almost normal after 6 weeks and normal at the age of 4 months. The time of observation is still too short to allow for any definit conclusions.

Acta Pædiatrica Skandinavica 56. 307–31* May 6"

CASE REPORT

# Disseminated Eosinophilic Collagenosis and Familial Eosinophilia

by STIG SPARREVOHN

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Since Loeffler [13] in 1936 described the now classical syndrome of transitory pulmonary infiltrates and eosinophilia, several publications have appeared about transi tional forms between Loeffler s syndrome disseminated eosinophilic collagenosis, pa narteritis nodosa and eosinophilic leukaemia. A special syndrome in childhood with recurring pulmonary infiltrates, hepatosplenomegaly leucocytosis and pronounced persisting eosinophilia and at the same tim a rather benign, chronic course was published by Zuelzer & Apt [21] in 1949 In this paper reference is made to a child who presented a complex of symptoms reminiscent of the syndrome described by Zuelzer & Apt This child presented an interesting family history and the eosinophils presented particular morphological features.

## Case Report

Girl, born 8.5.1964, no. 2 of 2. Uncompli cated pregnancy and birth. Birth weight 3450 g N neonatal diseases. Natural development Poor social conditions.

*1st admission*

Nov 11 t Dec. 17 1964 6 months old. Previously healthy 5 days coryza, fever increasing asthmatic respiration On the ad mission crepit tion over left lung was heard. She w treated with sulfamethoxipyridazin and diphenhydramin. A complicating bi lateral otitis wa treated with penicillin. Total recovery X-rays of the lungs 3 days after admission showed increased linear mar kings on both sides and infiltrat on the left side Ten days later bilateral pulmonary in filtrates and pleural effusion on the left sid Five days later the infiltrates had disppeared and the pleural effusion had de creased.

Blood tests showed nothing bnormal ex cept a slight eosinophilia of 625 per mm³ at the admission and some days later 19 per mm.

The case was diagnosed as asthmatic bronchitis, bronchopneumonia nd otitis media.

*2nd admission*

Oct. 7 t Nov 3, 1965. 17 months old. Apart from recurring coryza she had been doing well. Readmitted with the same symptoms as the first time Treatment the same plus ephedrin and lung physiotherapy Recovery was good but this time a marked eosinophilia of 18,400 per mm³ wa found. Because of few ascaris-like eggs in the stools he was treated with hexylresorcin and piperazin with no resultant evacuation of ascarides and no effect on the linical picture and the eosinophilia.

Repeated stool examinations for parasites or eggs were negative and there were no radiological signs of ascarides. Lung X-rays showed pulmonary infiltrat on the right side. ESR 5 mm, haemoglobin 10.3 t 11 4 g/100 ml, leucocytes 18,300–9990–16,200.

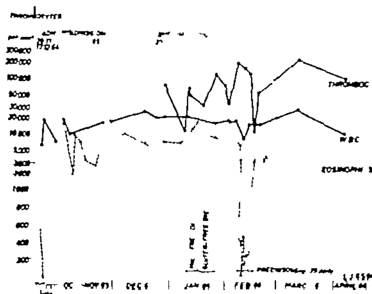


Fig. 1 Changes in the number of cells in peripheral blood during the 3 admissions.

Differential count showed an eosinophilia of 32–48–38%. Tuberculin reaction: negative. Urine-microscopy: normal. She was discharged in good health for later readmission for further examination.

### 3rd admission

Nov 27 1963 to Feb. 23, 1960 18 months old. Been in good health at home. Last 6 hours before admission developed the same picture of symptoms as on previous admissions. Treatment same. The eosinophil count at the admission was now 9000 per mm and it remained markedly increased.

The eosinophilic leucocytes represented a maximum of 62 % of the total leucocytes. An intermittent thrombocytopenia was found with a lowest thrombocyte count of 12 000 per mm. Concerning the change in the leucocyte-eosinophil and thrombocyt counts, see Fig. 1.

### Laboratory investigations

Haemoglobin 10.7–11.5 g 100 ml, MCV 80 MCHC 30 g/100 ml, bleeding time 3½ min, coagulation time 1½ min. ESR 24–47–17 mm, serumbilirubin 0.2 mg 100 ml, thymol turbidity 0 08, zinc sulphat 0 17 serum GO-transaminases 20 units (normal: < 40) prothrombin 72%, serum-electrophoresis: normal pattern. Immuno-electrophoresis on serum showed unspecific changes. LE-cells and LE-test antinuclear factor negative. No thyroid complement-binding antibodies or antibodies against thyroglobulin. No thrombocyte antibodies. Standard allergy cutaneous test were negative. Intracutaneous trichina test and trichinosis-complement binding-reaction negative. Examination of food antibodies on Ouchterlony plates with cow milk, haddock and egg was negative. A trial with elimination of gluten and milk for a period of one week each showed no change in the eosinophilia or effect on the clinical course. Repeated X rays of the lungs showed bilateral pulmonary infiltrates with migrating localization. Repeated stool examinations for parasites were negative and repeated urine microscopies showed nothing pathological, especially no erythrocyturia. Tuberculin test (Moro) was negative. X-rays of long bones of the upper and lower extremities and X-rays of the skull: normal.

### Special examinations

*Peripheral blood* "Shift to the left" of the neutrophilic leucocytes. Extreme eosinophilia with a "shift to the right" of the nuclear

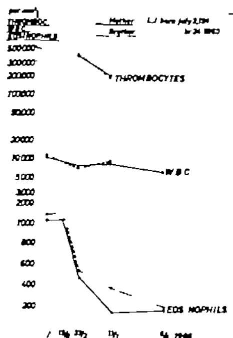



Fig. 1. Changes in the number of cells in peripheral blood of the patient mother and brother

segmentation of the eosinophilic leucocytes. Count of nuclear lobes of the eosinophilic leucocytes was done on 200 consecutive cells in leucocyt concentrat [17] and the mean lobe number was 2.59

*Bone marrow* showed no signs of leukaemia, no tumor cells, pronounced eosinophilia

*Skin window* according t Rebuck & Crowley [16]. A 6-hour experiment showed on the 4–5–6 hour coverslips striking eosinophilic emigration but the mean lobe umber w only 2.00.

*Liver biopsy*: Chronic inflammatory reaction in the portobiliary spaces. Eosinophilic leucocytes dominating the infiltration. No other pathological changes.

*Ski and muscle biopsy*: In the muscle tissue no changes suggesting periarteritis nodosa, but around many vessels inflammatory infiltration with granulocytes. Large macrophages, some eosinophilic leucocytes and lymphocytes were seen, mostly outside the walls of the vessels but in some places infiltrating the walls. A remarkable increase of mast cells in the neighbourhood of the vessels was noted In the skin infiltration with mononuclear cells some eosinophils and mast cells were found. The findings thus not being conclusive of panarteritis nodosa but strongly suggestive of some kind of a collagenosis.

Tentatively prednisone 5 mg four times daily was given for one week during which the eosinophil count in the peripheral blood fell to 44 cells per mm After discontinuing prednisone the eosinophilia immediately returned to the previous high levels (Fig 1). During the steroid administration the bronchial symptoms and the coryza were unaffected but she became listless and irritable.

*Outpatient observation* March and April 1966 showed persistent eosinophilia of 39 nd 26% with eosinophil count of 19 700 and 3075 per mm Otherwise she was doing well and had no upper respiratory tract symptoms.

*Family studies* The mother and the brother were found to have eosi ophilia of the peripheral blood. Skin muscle biopsy on both of them showed changes suggesting collagenosis. Perivascular focus with infiltration of leucocytes, fibroblasts, lymphocytes, some eosinophilic leucocytes and an increase of mast cells was noted. Findings in the family studies are shown in Table 1

Other maternal relatives could unfortunately not be studied but they were not known to have had respiratory complaints, exanthemata or allergic diseases.

## Discussion

Eosinophilia may be found in numerou diseases such as allergies parasitic diseases hematological diseases, mesenchymoses and first of all in panarteritis nodosa lthough eosinophilia in panarteritis nodosa is rarely found in childhood [9].

Eosinophilia is found, too in Loeffler syndrom in tropical eosinophilia tuberculosis, leprosy and scarlet fever It may be found in connection with various tumors

TABLE I *Family Studies*

| Members of the family | Peripheral blood: Eosinophils (per mm³) | Peripheral blood: Leucocytes (per mm³) | Peripheral blood: Thrombocytes (per mm³) | Serology (LE-test, RA test, ANF, electrophoresis) | Alkaline phosphatases and GO transaminases | Skin-muscle biopsy |
|---|---|---|---|---|---|---|
| Mother 21 yrs. old | 1 055<br>1 100<br>454 | <br>10,700<br>7 660 | <br><br>400,000 | Normal | Normal | Changes suggesting collagenosis |
| Brother 4 yrs. old | 1,330<br>1 462<br>6 3 | <br>10,900<br>7 700 | <br><br>4 4,000 | Normal | Normal | Changes suggesting collagenosis |
| Father 33 yrs. old | 188 | | | | | |
| Father mother | 182 | 13,300 | | | | |
| Mother father | 160 | | | | | |

such as tumors of the ovary and bronchogenic tumors. Finally eosinophilia has been reported as familial eosinophilia.

The diseases involved in the case described above are eosinophilic leukaemia disseminated eosinophilic collagenosis and familial eosinophilia.

Familial eosinophilia [1 2, 6 10 1* 14 18] has been reported in 15 cases in connection with examination of families of patients suffering from asthma asthmatic bronchitis recurrent upper respiratory disease, pulmonary infiltrates and eosinophilia.

Allergy was found in the family in 5 of the cases. In the majority of the cases the eosinophilia had been transferred through the mother The eosinophilia was of an intermittent type without any accompanying signs of disease

Disseminated eosinophilic collagenosis [8 15] is a disease characterized by hepatomegaly splenomegaly universal swelling of the lymph nodes exanthemata myalgias, subcutaneous inflammatory edemas eosinophilia involving many organ systems, focal interstitial degeneration and necrosis with predominating eosinophilic infiltration vascular changes varying from slight endarteritis to necrotizing arteritis The majority of cases described as eosinophilic leukaemia, are probably disseminated eosinophilic collagenosis.

Odeberg [16] reviewed the literature and came to the conclusion that the diagnosis of eosinophilic leukaemia could only be maintained in a single case He suggests that the diagnosis eosinophilic leukaemia should be restricted to cases with common clinical hematological and pathological findings, corresponding to granulocytogenic leukaemia but with a constant, pronounced eosinophilia in the peripheral blood and in the bone marrow with both mature and immature eosinophilic leucocytes until the occurrence of death or a change into a terminal myeloblast crisis. There should further be no signs of infectious, parasitic or collagenic disease which might be the cause of the leucocytic

proliferation found. Bousser [5] has published an extensive review on eosinophilic leukaemia and eosinophilic collagenosis. Engbæk *et al.* [7] have reported a fatal case of a 7 year-old boy: in this case the pathological findings resemble what we call eosinophilic collagenosis and the authors suggested the name "eosinophilia leukaemoides". In Scandinavia eosinophilic leukaemia has been reported by Thomsen & Plum [10], Jacobsen [11] and Bengtson & Nordenstam [3].

In 1949 Zuelzer & Apt [21] described a syndrome in children aged 18 months to 3 years characterized by a pronounced, persistent eosinophilia, hepato-splenomegaly with eosinophilic infiltration in the liver and in many other organs, lymphadenopathy, hyperglobulinemia and with a chronic benign course.

In 5 of the cases asthmatic bronchitis, recurrent upper respiratory disease and coryza as well as pulmonary infiltrations were found. Similar cases have been reported by Bass [3] and by Valledor *et al* [20].

In many ways the case presented here recalls the syndrome described by Zuelzer & Apt.

Our patient had recurrent asthmatic bronchitis, transitory pulmonary infiltrates, leucocytosis, persistent eosinophilia, intermittent thrombocytopenia and marked eosinophilic infiltration in the bone marrow. However she had no hepato-splenomegaly, lymphadenopathy or hyperglobulinemia. On the other hand she exhibited other interesting features. An examination of nuclear segmentation of the eosinophilic leucocytes of the peripheral blood was performed on a leucocyt concentrat [17]. The number of nuclear lobes was counted on 200 consecutive eosinophilic leucocytes. As in other eosinophilias [17] a so-called "shift to the right" was found with a mean lobe number of 2.59. At the same time a skin window experiment according to Rebuck & Crowley [16] was done. Here it was observed that the emigrated eosinophils of the inflammatory exudate were practically all bilobed with a mean lobe-number of 2.00. This might suggest that only the young eosinophils migrate into the inflammatory exudate. On no occasion had our patient had any exanthemata, dermographism or signs of urticaria pigmentosa. Her skin muscle-biopsy showed changes suggestive of collagenosis and liver biopsy showed eosinophilic infiltration.

Skin muscle-biopsy performed on the patient's mother and brother, who both had eosinophilia in the blood, showed similar changes, suggesting collagenosis. They had never had any signs of disease and had never been hospitalized. The eosinophilia had disappeared on later follow ups but familial eosinophilia was reported to be of an intermittent type. They had negative serological tests.

One of the cases reported by Zuelzer & Apt had a history and symptomatology similar to those of our patient. This patient had a sister who had a marked eosinophilia of the blood without any signs of disease. Cases of familial eosinophilia are likewise found on examining relatives of patients with allergic upper respiratory symptoms. There is apparently a gradual transition between familial eosinophilia, disseminated eosinophilic collagenosis and the syndrome described by Zuelzer & Apt. It might be a question of a familial hereditary, possibly autoimmune form of

reaction which in our case and in the cases reported by Zuelzer & Apt plays its role in early childhood—with a benign course


## Summary

Report was presented of an 18-month old girl with *recurrent asthmatic bronchitis*, recurrent pulmonary infiltrates leucocytosis persistent marked eosinophilia with a "shift to the right" in the nuclear segmentation in peripheral blood intermittent thrombocytopenia eosinophilic infiltration of the liver and bone marrow skin muscle-biopsy suggesting collagenosis, benign chronic course, no signs of allergy on common skin-tests and no food allergy. No sign of parasitic disease. The mother and a brother had transitory eosinophilia without signs of disease and skin muscle-biopsy on both of them suggested collagenosis. It was concluded that the patient exhibited *symptoms comparable* with those found in the syndrome reported by Zuelzer & Apt in 1949 combined with familial eosinophilia and possibly familial collagenosis.


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CASE REPORT

# Holt Oram Syndrome

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In 1960 Holt & Oram [5] described a familial syndrome featuring atrial septal defect, thumb anomalies and peculiar supraventricular arrhythmias. The presence in members of successive generations suggested dominant heredity McKusick [8] who described the second family proposed the term "atrio-digital dysplasia Zetterqvist [11] and Kuhn *et al* [6] in 1963 Pruzanski [9] and Lewis *et al.* [7] in 1964 Holmes (two families) [4] in 1965 Gall *et al.* [2] and Ehlers & Engle [1] in 1966 have published 8 further families all showing a clear dominant transmission.

Though the ostium secundum type of atrial septal defect (ASD) is the most usual heart anomaly ventricular septal defect has also been described in some instances [ 4 6, 7]. Exceptionally ostium primum ASD [4], patent ductus arteriosus [..0], and right ventricular hypertrophy without any septal defect [6] were present

The arrhythmia is characterized by a tendency to sinus bradycardia with nodal escapes and long P–R intervals Ectopic rhythms may also be present

The usual hand anomalies show hypoplasia or even aplasia of the first ray (thumb first metacarpal bone radial border of the carpus) on one or both hands, and even the radius itself. Extreme cases may present phocomelia [6 9]. Most often there is a finger-like distally placed triphalangic, hypoplastic thumb but its complete absence is also frequent. The first metacarpal bone is either hypoplastic or absent Pectus excavatum and hypoplasia of the glenoid fossa and shoulder girdle [1 2] may also coexist.

The present family eleventh in the world literature, has 4 typically affected members, mother and ll her children, with varied expression.

## Case Reports

### *Case 1*

Mother She is a 3.. year old woman. Her cardiac examination ( ausculation, ECG X rays) does not reveal any suspicion of congenital heart disease Her ECG (Fig. 1 A) is normal, except for slight prolongation of the P–R interval (0.2.. sec.).

Her hands (Fig 2) present hypoplastic finger-like thumbs, the left syndactylic to the index finger Radiographically the right hand has a thin first metacarpal and triphalangic thumb whilst there is no left first metacarpal, the left thumb having two rudimentary phalanges. There are also minor alterations in the radial side of the carpus.

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[1] receipt of grant Aid to the Research or Doctors, from the Ministry of Education and Science

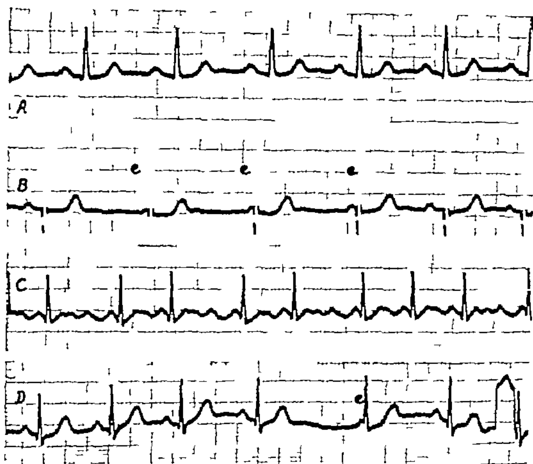



Fig 1 ECG of the affected members. A mother B eldest daughter C, son. D youngest daughter e nodal escapes For explanation, see text.

*Dermatoglyphs* She has 8 ulnar loops and 2 radial loops (one on the left thumb), total finger ridge count (TFRC) scores 87 Hypoplasia of the thenar area produces an atypical palm print with absence of the axial triradius on both palms *a*, *b*, *c* and *d* triradii are all present there exists a whorl under right *a* and a loop between left *a* and *b*

*Case*

Elder daughter 9 years old. She was operated upon in January 1966 to close an ostium secundum ASD

Her ECG (Fig. 1 B) besides right axis deviation and rsR pattern in V (typical for ASD), reveals tendency to sinus bradycardia, with some nodal escapes.

She has normal hands, both externally and roentgenologically

*Dermatoglyphs*. She has 3 whorls 2 radial loops and 5 ulnar loops on her fingers; TFRC scores 98 Both palms are normal except for the presence of a very high *t* triradius (position *t*") with *atd* angle of 90° on both hands.

*Case 3*

Son. 8 years old, he has been also operated upon in October 1965, for the closure of an ostium secundum ASD



Fig. _ Abov hands of the mother Below hands of her youngest daughter

His ECG (Fig. 1 C) reveals atrial flutter with changing 2 1 to 3 1 A–V conduction. His hands are normal.

*Dermatoglyphs*. He has 6 whorls and 4 ulnar loops TFRC scores 84. Right palm has double t triradius, being the distal one in a very distal (t") position, with *atd angle* of 75 Left palm has a normally placed (t") axial triradius (*atd angle* = 40°).

*Case 4*

Youngest girl 3 years old. Clinical and haemodynamic examination reveal a clear atrium secundum ASD

Her ECG (Fig. 1 D) shows the typical rsR pattern in V and occasional marked sinus bradycardia with nodal escapes.

Her hands reveal absence of the left thumb and a hypoplastic, syndactylic, triphalangic right thumb (Fig. 2).

*Dermatoglyphs* She has 6 whorls and 3 ulnar loops (including one on right thumb); TFRC scores 143. Palm prints are atypical, resembling her mother's; no t triradius is present on each palm; b and d triradii are present; no other figures exist. Karyotype is normal (46/XX)

Other members of the family:

The father has a normal heart, normal ECG and no hand anomalies. His dermatoglyphs present 10 ulnar loops, TFRC 47 and *atd angle* 45 (t position).

No other members of the family have been personally examined, but none of them has hand anomalies and only one has been said to have a heart (rheumatic?) lesion.

## Comments

This family is an example of the so-called Holt Oram syndrome with clear dominant inheritance and variable expressivity

Atrial septal defect is present in all three children.

Electrocardiographic abnormalities are present in the mother and her 3 children, the mother having long P-R interval both daughters having tendency to sinus bradycardia with nodal escapes, and the son atrial flutter

Hand anomalies are present only in the mother and her youngest daughter and all degrees of hypoplasia of the first hand ray are present finger like thumb (mother s right) syndactyly (mother s left and daughter s right) absence of the thumb (daughter s left) hypoplasia of metacarpal and first finger bones (mother s and daughter s right) triphalangism of the thumb (both s right) and absence of the first metacarpal bone (mother s left) There are also minor carpal anomalies in the mother

Palm dermatoglyphs are similar in the members with hand anomalies (mother and youngest girl) both lacking *t* triradius. The other two affected members have an abnormally distal *t* position (*t*''). All three children have several whorls on their finger tips while none is present on their parents this phenomenon accounts for the increase in TFRC for all children, particularly for the youngest (TFRC = 143) whilst the other two have similar values (98 and 84) to the mother s (87), but all of them higher than the father s (47) and midparent (67) values.

Dermatoglyphs have been found to be of great interest in congenital heart disease many cases show a tendency towards a distal position of the axial triradius (*t*' or even *t*'') [3 10] In Holt-Oram syndrome Holmes [4] and Gall [ ] have found a distally placed axial triradius, but two affected persons [ ] lacked it, similarly to our family s most affected cases.

Karyotype of one member of our family was, as expected normal, as karyotypes of other cases in the literature [_, 4 11].

## Summary

A family with four members—mother and her three children—bearing features of the so-called Holt-Oram syndrome is reported. Hand anomalies were only present in the mother and youngest daughter atrial septal defect was present in all 3 children, electrocardiographic aberrations were present in all 4 affected members.

Dermatoglyphs showed abnormal patterns in all patients.

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*Key words* Holt-Oram syndrome, atrial septal defect, dermatoglyphs.

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LETTER TO THE EDITOR

# Evaluation of the Forward Triangle Factor in the Newborn Infant

In *Acta Paediat Scand* no 1 1967 we reported a modification of the forward triangle factor used in the calculation of cardiac output from the first part of n indicator dilution curve In contrast to a factor value of 0 34 computed from studies in ad lts, we found the a erage value for newborn infant to be 0.23.

This figure was based on calculations by area-approximation, which is the standard procedure for dye-curve appraisal. Later an effort was made to confirm the proportion ality factor by using more refined analytical methods including planimetric assessment of the area under the curve. This has not only added substantially to the accuracy of the area calculation but also allowed more critical evaluation of the shape of the whole curve As a consequence of this we have found it necessary t discard 5 of the original 60 curves as being technically less ideal for the study The planimetric appraisal of the remaining 55 curves is graphically presented in Fig. 1 and resulted in a slightly higher a erage value for the forward triangle factor of 0.254. We consider that the difference of 10 per cent is important enough to bring this new analysis to your readers ttention.

*John S Hanson William Oh René A Arcilla Göran Wallgren John Lind*

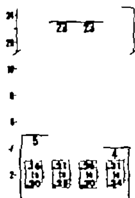


Fig. 1 Frequency distribution of forward tri angle factor *K*, calculated from dye-curves with left atrial injection and proximal aortic sampling in normal newborn infants.




Fig _ Frequency distribution of differences in cardiac output calculated by Hamilton re-plot method and forward triangle method using factors for the latter of 0.34 and 0.25.

Acta Paediatrica Scandinavica 56. 319–330 May 1967



# The Finnish Pediatric Society

Meeting Aug 1–2, 4 1966

(together with the Pediatric Society of West-Germany)

*G J ppich* (Göttingen). Neurological aspects in premature infants

*Reijo Neri* The congenital nephrotic syndrome—a hereditary disease

The congenital nephrotic syndrome (CN) has been suspected of being hereditary because of its familial occurrence In Finland CN is more prevalent than anywhere else more than half of the reported CN families are from Finland (57/103) A genetic study of the Finnish CN families has been carried out An initial series of 39 CN families was extended by finding 18 new evident families in the home localities of initial CN families and through the records of given hospitals. By investigating the family data of these sibships and by tracing their ancestries within 6–10 generations with the aid of the excellent population register vailable in Finland, following results were obtained, which argue in favour of autosomal recessive inheritance:

—The proportion of ffected siblings, corrected by different methods, is very close to the theoretical 0.25 (0.250 according to complet truncat ascertainment in families whose parents are married 1930 or later).

—The sex ratio of affected children is close t 1 (1 07).

—Sixteen remot consanguineous marriages and a great number of close or distant relationships between several CN parents were disclosed. Thus, 10 CN parents could be shown t be descendants of one and the same common ancestor pair living in the 18th century The fact that no first-cousin marriage ould be found among the CN parents is accounted for by the great rarity of first-cousin marriages in Finland at present Certain peculiar features of the Finnish settlement and population structure seem to explain these findings and, on the basis of utosomal recessive transmission, the abundant occurrence of CN in Finland.

*Jussi Vilska* Drug-ind ced renal disease in newborn rats

Two methods of producing nephrotic disease in newborn rats were studied Large sublethal doses of puromycin aminonucleoside were administered 1) t newborn rats, 2) to pregnant rats in the last trimester In the former severe nephrotic disease resulted in the newborn rats with growth retardation and reversible glomerular and tubular changes visible in light microscopy and electron microscopy A similar but milder ffect was btained by the second method. The renal disease of the newborn produced by these two methods showed complete healing; no definit thickening of the peripheral basement membrane was observed. In the discussion of the results it was emphasized that diffuse damage of the kidneys capable of leading t progressive disease cannot be produced in the rat foetus or the newborn rat owing to developmental immaturity of the kidneys.

*Gerhart Erdman* (Mainz) Immunological aspects of cow milk allergy

Owing to acquired sensitization of the human organism to the various foreign pro-

teins of cow s milk (casein alpha-lactalbumin, beta lactoglobulin, bo ine serum proteins) the intake of cow milk in any form may cause pathological manifestations due to cow milk allergy In paediatric practice disturbances of this kind are as yet of little importance. They can be recognized by xposu re and skin test with purified cow' milk proteins. From the standpoint of dif ferential diagnosis, the main alternative to be ruled out is lactose malabsorption which causes simila linical dist bance.

It ppears that immunological tolerance tow rd cow milk, which may principally be acq ired by early contact in infancy is of importance This iew is substantiated by statistical analysis of the time when the administration of cow milk began in a group of 139 children with eczema and in two control groups, one consisting of 139 children with pneumonia, the other consist ing of 139 children with otitis. On average, the children with eczema had come into contact with cow s milk later than the con trols. Furthermore the former group hardly contained any prematures or babies with poor weight gain. As compared with the control groups, the difference was statistical ly significant

*M Kekomäki J Perheentupa and J K Visakorpi* Protein intolerance with basic aminoaciduria

A formerly unknown type (*Lancet II* 813 1965) of basic (lysinuric) aminoaciduria, accompanied by gra e disturbances in the prot in metabolism, has proved t be not very unusual in the Finnish population. During the last two years, the quit unvari able clinical syndrome (intermittent vomit ing and diarrhoea dating back to increase of protein intake during the first year f life which later on change to nearly total aversion to foods of animal origin, short stature and hepato(-spleno-)megaly com bined with typical biochemical findings (basic aminoaciduria and a lowered capacity to urea synthesis manifesting itself as a periodic hyperammonaemia) has been diagnosed in ten Finnish children. The features of this syndrome the possible primary disturbance in the amino acid metabolism and the mana gement of the children with extra arginine were briefly discussed.

*K Launiala, A Pasternack, J Perheentupa and N Hallman:* Familial chloride diarrhoea ("congenital alkalosis with diarrhoea")

Since the first reports on congenital alkalotic diarrhoea were published by Gamble *et al.* and Darrow in 1945 about 10 cases have come to our knowledge from different places. During the last 5 years we have seen 9 additional patient with similar symptoms at the University Children Hospital in Helsinki; we call the disease familial chloride diarrhoea.

W have been able to follow ll our pa tients from the newborn period. The familial character of the disease is clearly established, more than one sure or probable patient having been born in three of our families. The disease presented itself before birth as hydramnios in the mothers of all our patients. Eight of the 9 patients were born before term. Loss of weight in the first week was excessive 25 per cent in one patient

Lack of meconium was recorded in 4 cases, abdominal distension developed during the first few days in 6 cases and intestinal obstruction was suspected on admission. It seems possible that a prenat l diarrhoea exists.

A definitive diagnosis could be made on the basis of the strikingly high Cl content of the faecal fluid. This remains later con stantly at about 150 mEq/l and exceeds the sum f K + Na. Extreme hypoelectrolytaemia, especially hypokalaemia and hypochloridaemia, developed in most of the patients, but periods of even severe metabolic acidosis occurred in the neonatal period, in contrast to the subsequent constant tend ency t metabolic alkalosis which gave the disease its first name

A very low Cl content in the urine has been considered characteristic. This finding was made in 6 out of 8 patients followed by

ns. The concentration varied from practically nil t a few mEq/l, obviously depending principally on the plasma level. Intestinal function was studied by intubation during homogenous fluid diet and constant infusions of salt solution, both containing PEG \ defect in the resorption of K was observed. The resorption f Cl was found to be defective as indicated by high chlorid concentration throughout the whole length of the gut. The name Cl malabsorption principally refers to this finding

Percutaneous renal biopsy was performed on 7 patients. The age of the patients at the moment of biopsy varied from one month t 4 months. Hypokalaemic nephropathy was observed in three of the youngest children. Juxtaglomerular hyperplasia was present t some degree in all but one of the cases. Heavy Ca-deposition in the kidney was rather constant finding In spit of varying glomerular destruction, advanced arterial and arteriolar narrowing was seen. In one child that came to autopsy extremely severe arteriolar changes were found, including, for instance alterations of the coronary arteries.

In two patients plasma renin activity and urinary aldosterone excretion were estimated (R. Veyrat Genèv ) Both were found t be levated, which constitutes definit evidence of secondary aldosteronism. The blood pressure was normal in all patients.

On the basis of the findings described above the following hypothesis regarding the pathogenesis is set forth. The basic defect is a failure of chloride resorption in the intestine. This leads to depletion of salt and w ter i.e stat of longstanding hypo-olaemia. The result is an increased activity of the juxtaglomerular cells, reflected by the juxtaglomerular hyperplasia and the increased renin activity observed. The out come is high activity of angiotensin, a possible cause of angiopathy and secondary hyperaldosteronism.

*G Wilhelm* (Frankfort am Main)· A nucleoprotein with collagen-precipitating proper ties

In the human and animal organisms a nucleoprotein was recently detected which brings about the conversion of trophocollagen to fibrinous collagen. The new substance is markedly superior to all others hitherto found with respect to the effect on the process in question. The fibrils formed show a perfect, normal microstructure The results of the reported studies seem to imply that the substance detected is precisely that which in the human and animal organisms effects the conversion f trophocollagen to fibrinous collagen. In patient suffering from liver cirrhosis and chronic nephritis, intra cutaneous tests with this nucleoprotein recovered from human tissue were positi e The reaction followed a pattern of tuberculin type It may be assumed that it will now be possible for the first time t isolate an utoantigen directly associated with the connective tissue In bacteria a simila nucleoprotein was demonstrated, which likewise possesses collagen-precipitating properties, although immunologically it differs distinctly from the nucleoprotein present in the human and animal organisms. In high dilutions, the nucleoprotein recovered from streptococci of groups A and C gave positive skin reactions of tuberculin type in patients who suffered or had suffered from rheuma tism. This reaction seemed to be highly specific. To the nucleoprotein recovered from tuberculosis bacteria children reacted differ ently depending on the size of the focus and the time of infection. The result of test hitherto performed on 300 hildren seem to warrant the conclusion that new methods for the diagnosis f tuberculosis, which take activity into account can be developed on this basis.

*P. F rauli:* Cytomegalovirus-infection and hepato-splenomegaly

*W. Künzer* (Freiburg i. B.): Hereditary haemolytic anaemia with post-splenectomy formation of Heinz bodies and mesobilfuscinuria associated with an abnormal haemoglobin

Case report of haemolytic anaemia associated with haemoglobinopathy, mesobilifuscinuria, and formation of Heinz bodies after splenectomy in a 4½ years-old boy. The splenectomy had been done at the age of 3 years under the tentative diagnosis of a hereditary spherocytosis. The normochromic anaemia was compensated before and after the operation; no blood transfusions were necessary. There was continuously a marked reticulocytosis even after splenectomy. Because of the following findings this condition may be classified as the above named haemolytic syndrome of which some 25 cases have been reported up to now: dark-brown urine, subicterus, pallor of the skin since birth, a high reticulocyte count before and after splenectomy, formation of Heinz bodies in the majority of the red blood cells after splenectomy, an abnormal haemoglobin fraction migrating in starch block and starch gel electrophoresis between haemoglobin A and haemoglobin F as well as a mesobilifuscinuria accounting for the dark discoloration of the urine. In our patient the Price-Jones-curve has been shifted to the right, the osmotic fragility of the red blood cells was found to be altered on both the hypo- and hypertonic side. Examination of the bone marrow yielded an erythroblastic hyperplasia. Several red blood cell enzymes and the erythrocyte porphyrins were only uncharacteristically elevated in accordance with the high reticulocyte count. The patient's haemoglobin exhibited an increased tendency towards oxydation and in heat denaturation there was a more heat labile haemoglobin fraction of 23 %. The findings correspond closely to the observations of other authors. Our patient's father, a brother and the more closely related family appear to be healthy; in the mother the findings show some evidence suggesting a "trait" type of this disease.

*U. F rahjelm:* Selective malabsorption of Vitamin $B_{12}$ (to be published)

*H. Brüster* (Düsseldorf): Effect of saturated and unsaturated fatty acids on blood clotting

After the administration of fat (25 g of butter per sq.m of body surface) which sometimes leads to an increase in blood lipids the levels of prothrombin, factor VII, thrombokinase and antifibrinolysin all show a rise resulting in an increased clotting tendency of the blood. This phenomenon has been observed in healthy subjects on a fatty diet but still more in subjects on a fat free diet.

In the experiments reported the maximum increase in serum triglycerides was attained about two hours after the administration of 25 ml of *Arachis hypogaea* per sq.m of body surface. After 24 hours normal values were again noted in subjects on a fat-free diet.

The triglyceride level was determined after extraction of the lipids with chloroform-methanol. Chromatography of the lipids was performed with $SiO_2$, after which they were eluated with gradually rising concentrations of ether in petroleum ether. Subsequently the lipids were treated with chloroform-methanol. Chromatography of the triglyceride fraction and the free fatty acids was performed using Amberlite I RA 400.

In a two-dimensional paper chromatogram the main constituents after saponification of the oil were oleic acid and linoleic acid. (Unsaturated fatty acid-oleic acid about 56 %, highly unsaturated fatty acid—linoleic acid about 26 %). The effect of the extract on the total clotting of blood was studied by thromboelastography.

It is obvious that a change in the activity of the factors promoting the clotting of blood seldom occurs in healthy subjects. The situation is different when tolerance

tests are performed on patient with haemophilia. In the plasma thrombokinase formation test these patient showed an increase in total plasma thrombokinase activity which seemed to be indicative of an increase in the activity of the plasma lotting proteins.

Investigation of the coagulation-inhibiting factors revealed an increase in the activity of antithrombin II and antithrombin IV whilst the activity of antithrombin III was found to be inhibited.

The effect of the administration of *Arachis hypogaea* on the blood plat lets was striking Two hours after administration of the oil the blood platelets exhibited a decrease in number t bout 60% of the initial value

Apart from the decrease in number f the platelets, their ADP-induced adhesiveness showed change. The adhesiveness was en hanced by the addition of highly unsaturated fatty cids, e.g linoleic cid. The strongest effect, however was obtained by the admin istration of saturated fatty acids in combination with an unsaturated fatty acid (oleic acid).

Furthermore, after the administration of *Arachis hypogaea* extracts a change in the f brinolytic potential was clearly discernible Both the profibrinolytic time and the euglobulin lytic time were definitely shortened. The antifibrinolytic time was prolonged.

The effect of the administration of lipids on the total clotting system of the organism was also reflected in the change that took place in capillary permeability The number f petechiae was reduced not only in the vascular forms of purpura but also in the forms due t thrombocytes.

Further in estigations learly showed that the heparin-induced lipid learance remained functional during administration of the oil. The heparin released from the mast cells and the vascular organs activates the clear ing mechanism by mobilizing lipoprotein lipase

From the results the conclusion was drawn that the turnover rat of the plasma lotting proteins was enhanced and the f brinolytic activity inhibited. Furthermore the blood platelets were clearly influenced; they decreased in number lthough at the same time their release f plat let-specific clotting proteins was increased. That protracted administration of oil would result in early onset of sclerosis need not be anticipated, since lipoprotein lipase is released in normal amounts and no inhibition of lipolysis occurs.

*H Dost* (Giessen) Th lectronic analog-computer in th interpretation of blood curves

*Ole Wasz Höckert* St dies on the cries f newborn and young infants

The cry studies were started in Helsinki 1961 by Wasz Höckert Valanne Vuoren koski, Partanen and Michelsson. Using the sonographic method typical signal groups in newborn and infant were nalyzed and the bility to identify these signals was studied. A fruitful co-operation in Stock holm with Prof. Lind, Prof Fant and his group was organized from 1963 as they had been working with physiological aspect of the first cry also using the sonographic method. Now this whole team, which works in Stockholm, Oulu and Helsinki has en larged co-operation with pediatricians broad (Paris and New York).

W ha been able with a large material biostatistically t prove significantly the existens of four signal situations the first cry the hunger cry the pain cry and the pleasure cry Additionally large materials are partly published, partly under prepara tion concerning brain damaged children, chromosomal anomalies (Down, Maladie de chat). The asphyxia syndrome is subjected t a special study

The group has been able t show that not only the normal signals mentioned can be recognized by trained personel working with newborn and infants, but also that such distinct pathological cries as brain damage and hydrocephalus can be recognized as statistically proved, by neonatologists, pediatricians and children nurses.

*C. E. Räihä* Measures aimed at reduci g perinatal mortality and the frequency of premature birth

*Marjatta Kannas* Cerebrospinal fluid studies in newborn

The colour pigments of the cerebrospinal fluid of 94 newborn infants were studied spectrophotometrically A flat spectrum, i.e. one without any haemoglobin components, wa found in 30 cases. In 29 cases the peak of the curve fell in the bilirubin area of the spectrum (490–390 mm) This phenomenon bowed no direct correlation with the serum bilirubi val es. Spectra indicati e of fresh blood i.e with oxyhaemoglobin peaks at 578 and 54 nm and with their sum at 415 nm, were observed in 35 cases. When arti ficial bleeding was involved, the spectrum returned to normal in only one or two days. In cases of essential haemorrhage the spec trum showed typical changes for many days, owing to the presence of other haemoglobin—colour pigments, .e. methaemoglobin and bilirubin.

The spectrophotometric results coincided with the autopsy findings, but the present material is scanty and from the standpoint of prognosis this method of investigation seems to be of little value.

*E Schmidt* (Köln) On the dependency of pyridoxin in newborns

*T omas Pelto en and Leo Hirro en* The fetal bronchial fluid

The fluid in the fetal respiratory passages *originates in the lungs and respiratory passages*, and is not the same as the amniotic fluid. The following relative concentrations were observed. *Glucose* Maternal serum > fetal serum > amniotic fluid > bronchial fluid. *Cholesterol*. fetal serum > maternal serum > amniotic fluid = bronchial fluid. All these relationships were observed regardless f fetal size *Creatinine* in the smaller guinea pig fetuses the content in the bronchial fluid was higher than in the blood, but in the larger fetuses the ratios were reversed. Probably the elimination of creatinine occurs more readily in the late fetal stages. The variations in concentration of the abovementioned substances in the different liquids depend also on the species of experimental animal. As the concentrations of the various substances in the bronchial fluid differ from those in the blood, it can hardly be assumed that the liquid develops by one single process such as filtration from the pulmonary circulation. Before the alveoli open, the pressure in the pulmonary artery is high. In this stage conditions are favourable for a shift from the blood circulation toward the respiratory passages whereas the pposit holds good fter the first breath has been taken. After aeration of the alveoli, the bronchial fluid remaining in the respiratory passages is normally absorbed into the pul monary circulation.

The composition and possibilities for resorption of the fetal bronchial fluid after aeration of the lungs perhaps constitute the essential factors in ol ed in the development of hyaline membrane disease.

*Dietrich Knorr* (München) Determination of urinary testosterone and its significance in the diagnosis of maturati n disturbances in boys

Urinary testosterone was determined in 204 healthy male children and adolescents using the isotope technique of Vermeulen & Verplancke.

After the eleventh year the curves for the mean values and the median values rose abruptly The rise was more abrupt than *the rise of the curves for* neutral 17 ketosteroids. Since the latter represent the sum of the metabolites of the adrenal and gonadal androgens, they yield no reliable information concerning the biological activity of the secreted androgens. In order to corroborate this statement two clinical cases were described.

A 5-year-old boy with familial pubertas praecox exhibited fully developed secondary sex characters. The 17-ketosteroid level was *1.5* mg/day which is t the upper limit of the normal range for this age The secretion

of testosterone was 32 µg/day which is within the normal range of adolescents. In this case gonadarche had already set in but not adrenarche.

The opposit situation is exemplified by a 16-year-old boy who exhibited definite secondary sex characters in combination with eunuchoid height In this case the 17 ketosteroids were 4.9 mg/day which is within the lower range of the normal fo the age in question. The testosterone secretion was 5 µg/day which corresponds to the prepubertal level. Thus, adrenarche had taken place whilst gonadarche had not yet begun.

*Leena Tuuteri* Cerebral palsy in Finland

T evaluate the needs f medical and educational rehabilitation the incidence of CP was investigated in an area corresponding t population of 1.5 millions. Questionnaires were sent t the CP-clinics and to institutes of mentally defectives with questions concerning the grade and type of the motor handicap, the incidence of mental retardation, of visual and hearing impairment, of convulsions and of speech defects. The incidence of CP-children ranged in the different parts of the investigated area from 1 7 t 2.0 for 1,000 live born children, which is in agreement with earlier investigations made elsewhere.

The need of special clinics in connection of all children departments in the central hospitals is stressed. These subcenters ought to have the possibility to send their difficult cases to regional centers where a more complet team of specialists would be work ing

*Lea Ylppö* The CP-problems from the viewpoint of Children Castle (Helsinki) and th International CP committee

*O Kuusto* Treatment f CP-children in a central hospital.

*Cosse L* [illegible] Aspects in Treatment of Cerebral Palsy by Medical Exercises

A CP-child s motor performances are not only trained by means f medical exercises but his sensory functions are also stimulated at the same time. The stimulation in particular is important t the young and to the youngest children, and it serves the purpose of giving them experiences they are—because of their disability—not otherwise ble to obtain. Later on and according to the CP-child' motor and mental maturity training of useful performances, f.ex. walking, self care etc., is added to the training program. In many cases, periods f a few month intensive exercising—especially at short and suit ble intervals—seem to be the most practical and sound way of treating CP-children at preschool age

*Hans Åkerblom Tapiola*

# The Danish Pediatric Society

Meeting Sept 8 1965

*N J Brandt:* The Frequency of Carriers f Hereditary Galactosaemia in Normal Population

As judged by the number of newly-diagnosed cases, the incidence of hereditary galactosaemia ppears t be of the order of 1 70,000 in Denmark, Britain and U.S.A. It is, however bvious that a number of cases are overlooked. As this disease can be diagnosed promptly and treated effectively t is important t attempt to obtain a true picture of the incidence of the disease

By determining the incidence of heterozygotes in a normal population, it may be possible t obtain better estimate of the

genuine incidence of the disease. The mode of inheritance in hereditary galactosaemia is autosomal and recessive. Both of the parents of the patients are heterozygotes. Heterozygotes are usually presumed to be clinically healthy but, by quantitative determination of galactose-1-phosphate-uridyl transferase (Gal UT) in haemolysed blood heterozygotes and homozygotes can be differentiated. Heterozygotes have approximately 50% of the enzyme concentration of homozygotes and, with the technique which was employed (N. Tolstrup's spectrophotometric method) the overlap between the two groups is 0.4%.

Among a total of 54 normal individuals, 13 were found to have enzyme concentrations which were definitely in the heterozygote limits. In 11 cases, it proved possible to undertake family investigations which revealed that either one of the parents or that one or more of the siblings or children had low enzyme concentrations similar to the probands. These 11 individuals could thus not be classified as normal homozygotes but must be presumed to be heterozygotes for hereditary galactosaemia. These presumed heterozygotes had an average G 1 UT concentration of 46% of that of normal homozygotes.

If the heterozygotic condition is considered to be definite in these 11 individuals the heterozygote frequency becomes approximately 1 50 and thus the frequency of the mutating gene 1 100. The frequency of the disease calculated from this is 0 01 – 0 0001. The frequency of hereditary galactosaemia should thus be 1 10,000 live-born infants. Inexplicable population-genetic conditions may however affect the calculation which, for this reason alone, must be accepted with reservation. The possibility cannot be excluded that more than one pair of genes control Gal UT.

Thus, the calculated frequency of the disease is 1 10,000 as compared with the finding of 1 70,000.

After this work had been concluded, R. G. Hansen *et al* (*Proc Soc Exp Biol*, *115* 160, 1964) and E. Beutler *et al.* (*Lancet*, *I* 353, 1965) published the results of similar investigations in U.S.A. Thus, three independent population investigations have been undertaken and all seem to indicate the heterozygote frequency for hereditary galactosaemia to be approximately 1 50 to 1 65. It thus appears to be justified to conclude that the disease of hereditary galactosaemia is still underdiagnosed to a very considerable extent.

The investigation: "Galactose-1 phosphate-uridyl-transferase" is published as a thesis, Munksgaard København 1966.

Discussion

*B. Friis-Hansen* asked whether Dr Brandt had found the so-called "Duartevariant" (Beutler *et al.*)

*N. J. Brandt* I did not find evidence of so-called "Duartevariant" in any case in the family investigations I undertook. From Beutler's preliminary communications, it is not possible in my opinion, to conclude with certainty that a new variant is concerned. It may well be anticipated in advance that there are several alleles for Gal UT locus.

*E. W. Flensborg* asked whether Dr Brandt's finding of raised Gal UT in mongolism had been confirmed by other authors.

*N. J. Brandt* My investigations in patients with Down's syndrome have been confirmed from many sources. The possibility has been discussed whether the Gal UT concentration in whole blood was solely caused by an abnormal leukocyte metabolism. It is, however definite enough that the Gal-UT concentration is raised both in erythrocytes and in leukocytes. Whether the hypothesis concerning the localization of a gene for Gal UT in chromosome no. 21 holds, is, however not yet elucidated.

*B. Zachau-Christiansen* Investigation of Infantile Mortality in Rigshospitalet

In the present century the Danish infantile mortality has fallen from 134 per thousand to less than 20 per thousand which figure is only surpassed by Holland and

Sweden. These countries have incidences f prematurity which are lower than the Danish figures by 5.9%.

In follow-up investigation of 9,280 infants born in Rigshospitalet Copenhagen, in 1959–61 where the incidence of prematurity is 2½ times greater than the average in the country as a whole 202 infant were stillborn and 435 died within the first year of life and, of these, 372 died in the first week of life The perinatal mortality was thus 78.2 per thousand, the mortality in the first week of lif 41 per thousand and, among these in 25 per thousand, neonatal asphyxia or telectasis were the causes of death. In the remaining infants, the mortality was 6.9 per thousand (entire country during same period: 7 4 per thousand)

Premature infants had first week mortality of 272 and according t the average Danish figures for the individual premature weight groups, approximately 300 should be anticipated. The lat infantile mortality for premature infants and both the early and the lat infantile mortalities for maturely born infant were below the verage figures for the country as whole (22/26 5.5/5.8 and 4.4/8.7). Two thirds of the premature infants who died from asphyxia/atelectasis/respiratory distress syndrome weighed < 1,500 g.

Among the deaths due to deformities in infancy (7 1 per thousand) and post mortem examination was conducted on practically all of the dead infants, 21 deaths due to cardiac conditions and 17 deaths due to conditions amenable to paediatric surgery are emphasized. All of these children, apart from those who died from respiratory distress, were investigated or submitted t operation in paediatric cardiac and/or paediatric surgical special departments and unexpected death occurred in only one case of bnormal origin of the left coronary artery

Twenty four infants died from infections, 22 of them after the first month f lif Fifteen of them were found dead and 13 of these were between one and six months. These were either cases of sudden unexpected death or must be considered to indicat that there are still reasons to be on guard against signs of apparently slight illness in infants. Medical help had only been summoned in two out of the 15 cases before death occurred and one of the children was actually dying when help arrived.

DISCUSSION

The question of whether intracranial haemorrhage or pulmonary atelectasis is the primary cause of death in many premature infants was discussed (*J Vesterdal B Friis Hansen B Zachau-Christiansen*) and reference was made to the work by Inge Tygstrup on autopsy findings in infant dying in the neonatal period.

Further the treatment of the "respiratory distress syndrome was discussed, including fluid therapy and, in particula positive pressure chamber treatment and tracheal int bation with a thin tube connected with a respirator (*B Friis H nsen, H Dyggve B Zachau-Christiansen*)

A question from *G Englsson* (Lund) concerning the significance of postmaturity for the infantile mortality could not yet be answered from the present material.

E. *Hippe* Megaloblastic Anaemia (Imerslund-Gräsbeck) in a One-Year-Old Boy Investig tions f $B_{12}$ Absorption

(Published in *Acta Paediat Scand* 55 510 1966).

DISCUSSION

*H Dyggve*. In the case presented by Sievers, the possibility of retarded ment l development was suggested. Is t known how these children develop later?

*E Hippe* In a follow-up investigation undertaken by Imerslund & Bjornstad, when the ages of the patient were 7 t 24 years, the growth and development had been normal and, in particular it was not possible t demonstrat mental deviations.

*C J Sievers* Subsequent mental development in the hild I described proved to be complet ly normal.

*Å Villumsen a d B Zachau-Christiansen:* **Alterations in Position of the Testes during th First Three Years of Life**

During the period 1959–61 approximately 9 000 pregnancies and deliveries were fully recorded in the Maternity Departments in Rigshospitalet Copenhagen The infants born were examined on the first and fifth days of lif Approximately 75% were examined again at the age of one year and approximately 50% at the age of three years t the hospit l

*At birth* 4.1 % of the boys showed incomplete descent of the testes, viz., 17.2% of the premature infant and 1.8% of the mature infants. The material comprised a total of 209 boys of whom 60% were premature. The incomplet descent of the testes was bilateral in 118 boys (73 % premature) and unilateral in 91 cases (44% premature)

By the *f fth day f life*, 43 boys had died. Out of the remaining 248 testes which were incompletely descended at birth 39 had descended by the fifth day

At the *age f one year* 52 cases of incompletely descended testes out of the 209 incompletely descended testes in 138 boys at birth had now descended spontaneously Thus approximately 75% f the testes had descended spontaneously the majority in premature infants. At this age incomplete descent f the testes was present in approximately one per cent

At the *age of three years* the descent was not found to be more complete than t the age of one year On the contrary signs of *ascent* were observed in occasional cases.

The pproximately 5,000 boys in the material were therefore, reviewed in view of signs of possible ascent of the testis. In a total of 69 boys, 87 testes had apparently moved upwards between the first and third years of life. In a group of boys seen both at the first and third years, half of the testes which were ascending t the age of one year were found to ha e descended again at the age of three years. The greatest incidence of ascending testes were found by the least trained examiners. This incidence of spontaneous ascent of the testis is, therefore unexpectedly high, but the uthors find it probable that the phenomenon does exist This might explain why in this material, a lower incidence of incomplet descent of the testes was found at one year (1%) than in in vestigations on Dani h schoolboys (1.5% excluding retractile testes) In 12 boys at the age of three years, 14 ectopically placed testes were found (over the inguinal ligament), and 11 of these had been found in the scrotum at birth.

### Discussion

In the discussion it was emphasized that the material did not permit conclusions as regards the time f treatment and the method of treatment for incomplete descent of the testis.

## Meeting Oct 14 1965

### Cerebral Paresis

*P Plum* Course and treatment of cerebral paresis illustrated by tw films and lecture

## Meeting Nov 10 1965

*C. E. Mabeck.* **Light Treatment with Particular Attention to Upper Respiratory Infections and Behaviour Problems in Children**

(Published in *Ugesk. Laeg* *128* 223 1966.)

*Sv Hesnild* **Money as Ther peuti Aid**

By way of introduction, reference was made t a recently published work by the British paediatrician, John Apley: Family Patterning and Childhood Disorders (Lancet, 1963)

where among other things, attention was drawn to the therapeutic effect upon bet behaviour difficulties of children and parents produced by placing reasonable financial resources at the disposal of the family

A problem family such as this is reviewed. The family was kept " bov water" for nearly three years by means of economical support of pproximately 2,500 Danish crowns. In Apley words, the target is to prevent family with problems from becoming a problem family

Detailed review of this family' case histories is refrained from as the department in the past months, has just established con tact with the most recent member f the social welfare team: the family adviser. *Instead, the newly-qualified family adviser* will present his experience from six months practical work.

*Folmer Tyrsted* (Family adviser): The family advisory service commenced on April 1965, the bject being that the family adviser should replace the previous supervisor In Copenhagen, family advisers are employed full-time and each has pproximately 52 families to supervise H is expected to have time t contact schools, spare-time clubs, nursery schools and all the institutions which may be involved in patching up a broken family Supervision is carried out by a specialist group consisting, in Copenhagen, of a psychiatrist, a psychologist a social worker and a lawyer

*A* I*de* D ta Analysis f the P tient M terial in the Coast Hospital in Refsnæs

This department deals with electively admitted patient with behaviour difficulties and environmental neuroses. The analysis shows that at any time, "sibling sets" con *stitut one third of the population of the* hospital which emphasizes the relieving effect which hospitalization such as this frequently has for the entire family Ad missions such as these thus, to a greater extent constitut help for a family in need than actual treatment of the hildren con cerned.

## Meeting Dec. 14 1965

Meeting with ladies in Domus medica. "J panese Christmas Party"

## Meeting Feb 10 1966

*J Vesterdal and Th. Rosendal:* Development of Centres f Ossification in Children with Low Birth Weights

In Sundby Hospital, Copenhagen, an in vestigation was undertaken on the development of the centres of ossification in 83 newly-born infants who weighed ...500 g or less t birth. In eight, calculations of the duration of pregnancy were very uncertain I the remaining 75 cases, development of the distal centre of ossification in the femur and the proximal tibial centre were only poorly correlated with the weight. Correla tion between the development of the centres f ossification and the gestational age was somewhat better From this investigation, it may be stated that if the femoral centre of ossification present the gestat onal age of the child, calculated from the first day of the mother last menstrual period is more than 231 days and if the tibial centre is also present, then this period is more than pproximately 248 days. If both centres are bsent, the gestational age is less than 297 days. In this material, there was no definit sex-difference but such a difference has been demonstrated by other authors in extensive materials, girls being found to be more developed than boys.

Discussion

*B Friis-Hansen* What are the conditions in dysmature infants?

*J I sterdal* These infants have low birth weights but relatively high gestational age In our material, development of the centres of ossification in such infants corresponded more to the age but the correlation was not good.

*S I estermark and P A Krasilnikoff:* Acute Dyspepsias in Children

A material of 476 children with acute diarrhoea was presented. In 96 of them, pathogenic intestinal bacteria were demonstrated in the faeces, in 52 cases other cases of diarrhoea were present in the family or immediate surroundings, 169 patients had simultaneous parenteral infection, in 20 cases the diarrhoea occurred in connection with alterations in the diet while in 139 cases no cause for the diarrhoea could be demonstrated. Among the 96 cases with pathogenic intestinal bacteria, there were nine with Salmonella infections of the gastro-enteritis type one case with infection with paraty phus B two with bacillary dysentery and 84 with coli infections. Sixty-seven per cent of the material were in the age group 0-1 year The group with pathogenic intestinal bacteria consisted predominantly of infants. No clinical difference could be demonstrated between the different groups. Fluid and electrolyt disturbances occurred most commonly in the youngest age groups and these infants also took a longer time to recover than slightly older children. Etiological subdivision is frequently uncertain in cases of acut diarrhoea in children

Discussion

*J I asterdal.* It is surprising that among 476 children with diarrhoea of varying sever ity originating from some of the worst districts in Copenhagen including barracks for the homeless, only one death occurred. This is probably primarily due to the fact that the population is in such a good stat of nourishment secondly because medical advice is sought quickly and, thirdly be cause the patients received adequat treat ment with fluids and electrolytes in hospital.

I do not consider that all cases of diarrhoea should merely be called diarrhoea and nothing more but that etiological subdivision should be ttempted. It is obvious, however that in a great number of cases we are unable to elucidate the etiology

*P Plum* All cases should not be termed diarrhoea but attempts should be made to subdivide the material according t the etiology If this is unknown, further attempts hould be made.

*Torben Iversen*

Acta Pædiatrica Scandinavica 56 331 May 1967

## BOOK REVIEWS

*W Fischer and H Weinland* Stoffwechsel der Galaktose und ihrer Derivat

Monograph in the series f Biochemie und Klinik, edited by G Weitzel, Tubingen, and N Zöllner München. George Thieme Verlag, Stuttgart 1965. 205 pages, illustrated. Price: DM 59

The volume is up to date and well filled with facts. The occurrence of galactose is thoroughly discussed as is its normal and pathological metabolism. The last sections deal with compounds closely related to galactose The material is organized in a logical manner rendering the information looked for easy to find. By way of Forsythia pollen and snail galactogen the authors pass t mammalian biochemistry where galactose and ts compounds are abundantly repre sented, in the mucus of the digesti e and urinary tracts, in specific blood group substances, intrinsic factor chorionic gonadotropin, milk of course sweat et The meta bolism along the Leloir pathway is clearly described. The authors actualize some details of particular interest for the clinician. The galactocerebrosides are synthesized from about the middle of the foetal period. The synthesis increases rapidly during the neona tal stage and the galactocerebrosides reach concentration in the brain bout 20 times higher than that of the glycocerebrosides (which, however are preponderant in pathological cerebroside accumulation) Since ga lactose can be made from glucose there should be no reason to relat this to the rich supply of lactose in the food nor t the high activity of the lactase in the gut during this age period. The latter factors may instead be of importance for securing the glycogen stores f the liver where bsorbed galactose is effectively trapped. Insulin influences the flow of galactose into the tissues but galac tose does not lead t release of insulin. The chapter dealing directly with clinical problems is short but then the title of the book does not imply long clinical excursions. The met bolism in congenital galactosemia is disturbed by the intracellular accumulation of galactose 1-phosphat which possibly lowers the level of inorganic phosphat and ATP The enzyme galactose 1-phosphat / glucose 1-phosphat uridyltransferase, the activity f which is lacking in the homozy gote, has a markedly lowered activity also in the heterozygote although not low enough to give clinical symptoms. Brandt valuable but not yet confirmed, observation that patients with trisomy for chromosome 21 have around 40% higher activity of the enzyme than normal individuals is referred to By feeding high concentrations f galactose to experimental animals a condition resembling galactosemia is produced with changes in eye lenses, liver and kidneys. Observations which could be of some linical importance—for the heterozygot mother with a homozygote foetus—are those show ing foetal damage when animals are fed large amount of galactose early in preg nancy The clinician does not find details of direct interest on every page of the book but the sections containing such form a nucleus enwrapped in layers of informative material. With ts long list of 3123 references and its clear concentrat of these the book will be found useful by anyone interested in galactose

*Birgitta Werner*

ANNOUNCEMENT

# International Course on Recent Advances in Neonatology Athens Greece. Sept 15–20 1967

This course will focus on recent advances in the care of newborn infants Emphasis will be placed upon clear succinct presentations of practical value in the care diagnosis, and treatment of diseases of the newborn patient. Ample time will be allowed for discussions with the American and European Guest Faculty *Host and Honorary Chairman.* Dr Spyros Doxiadis Chairman, Institute of Child Health, Athens, Greece

*Participants* Dr M. Cornblath, Professor of Pediatrics, University of Illinois Medical School Dr J Lucey Associate Professor of Pediatrics, University of Vermont College of Medicine, Dr S Gellis, Professor of Pediatrics, Tufts University Medical School Dr T Oliver Professor of Pediatrics, University of Washington Medical School, and Dr R. Smith Professor of Pediatrics, University of Florida Medical School

In addition to the American faculty a distinguished guest faculty from European medical schools will participate.

The tuition fee for this five-day course (-5 hours) for doctors from Europe and the Middle East will be $50 00 For further information write to Dr T VALAES, The Queen Anna Maria Institute of Child Health Goudi, Athens 609 Greece.

# The Vienna Meeting on Pediatric Neurosurgery

will be held October 11–13 1967 in the Congress-Center of the Wiener Hofburg (Vienna Imperial Castle)

The Vienna Academy of Medicine was ordered to organize this meeting *A* Main Topics. 1 Hydrocephalus .. Subdural effusions and hematomas in childhood 3 Intracranial space occupying processes in childhood. 4 The neuropathological problems in early childhood. *B* Fireside-Conference Deadline for submission of papers and Fireside-Conference is May 31 1967

Submissions are to be sent to: Vienna Congress on Pediatric Neurosurgery Vienna Academy of Medicine c/o Miss Ursula Binam, Alserstraße 4, A 1090, Wien 9 Austria (Tel. 4_04 10 )

Acta Paediatrica Scandinavica 56, 333-343 July 1967

From the Outpatient Clinic for Teenagers, Samfundet Folkhälsan, Helsingfors, Finland

# The Problem of Dyslexia in Teenage

by M. FRISK, E. WEGELIUS, T TENHUNEN O WIDHOLM and H. HORTLING

It has been indicated that specific reading and writing difficulties occur in 10–15 per cent of all children who enter school [7] and in 2–15 per cent of all adults with a normal intelligence [10]. It seems to be a tenable statement that if relatively mild forms are included, 10 per cent of all children suffer from dyslexia [5]. As the main cause of this condition a hereditary disturbance of certain neurological functions has been indicated [5] Furthermore brain injuries may play a part [6]. In an investigation performed in Czechoslovakia [8] three main etiological groups were distinguished. The largest group showed various forms of encephalopathy the second largest group a family history of dyslexia sometimes in combination with encephalopathy The smallest group consisted of individuals who could not be classified or who were neurotic. Dyslexia may cause problems at school and secondary neurosis [1 6].

The problem of dyslexia was subjected to study at the Outpatient Clinic for Teenagers, Samfundet Folkhälsan, partly in order to ascertain how reading and writing difficulties influence the school situation and the psychic condition, partly in order to elucidate the etiological background. The not quite adequat term dyslexia is used here to denote the syndrome of specific writing difficulties The criteria by which this syndrome was distinguished from other reading and writing difficulties will appear in the text.

## Material

The present series consisted of 41 girls and 81 boys. The majority came from the three lowest grades of secondary school, but strikingly many attended the higher grades and the gymnasium. Only some subjects came from other schools or after they had left school. The girls were on average somewhat older than the boys; the age group >17 years was most frequently represented. The boys were rather evenly distributed over the groups 10–13 years, 14–16 years and >17 years. (Table 1).

## Results

The material was divided into two groups; one group with a hereditary predisposition to dyslexia and another group without known hereditary

### *Physical observation*

*Anamnestic data relating to the development of the central nervous system* A history of primary enuresis until after the age of 5 was common. This disturbance as well as speech difficulties had occurred more

TABLE 1 *Material*

| | Girls | % | Boys | % |
|---|---|---|---|---|
| *School and grade* | | | | |
| Sec. school grade I III | 18 | 44 | 34 | 4 |
| grade IV | 3 | 7 | 12 | 15 |
| grade V | 7 | 17 | 10 | 12 |
| Gymnasium | 10 | 24 | 15 | 19 |
| Primary school | 1 | 2 | 5 | 6 |
| Other school or no school | 2 | 5 | 5 | 6 |
| *Ages* 10–13 years | 8 | 20 | 4 | 30 |
| 14–16 years | 14 | 34 | 29 | 36 |
| ≥ 17 years | 19 | 46 | 28 | 33 |

frequently among the boys. In the group with genetically determined dyslexia poor motor performances, characterized by instability and clumsiness and a poor drawing and writing skill had often been observed early in life. These symptoms were however also common among those boys who had no family history of dyslexia. (Table 2)

*Neurological observations.* Pathological electroencephalographic findings, mostly transient diffuse changes, mainly encountered over the whole convexity and strikingly often over the anterior and central parts of the brain, were noted in 47 per cent of all cases. High frequencies was found in the group with no dyslectic heredity and among the boys with genetically determined dyslexia. The lowest frequency was found among the girls with a family history of writing difficulty (Table 2). In 17 cases the changes of the EEG were regarded by the consulting EEG specialist as typically postlesional. Apart from the clearly pathological electroencephalograms, 12 were regarded as uncertain borderline cases.

Lefthandedness was not very common. More striking was the occurrence of various speech disturbances, e.g. stammering, muddled speech, etc. which were most frequent among the boys with genetically determined dyslexia. A noteworthy finding was a motoric disturbance resembling dysdiadochokinesia observed on alternating supination and pronation of the hands. These movements were irregular and awkward and were often accompanied by involuntary movements. In the group with hereditary dyslexia this symptom was noted in about half the cases, but disturbed motoricity was also frequently observed in the group with no hereditary involvement.

*Observations on the physical development.* In respect to physical development deviations were observed in the form of either advanced or delayed bone age, i.e. a plus or minus deviation by $\geq 1\frac{1}{2}$ years from the chronological age, or early or late menarche, i.e. at the age of 10–11 or 15–16 years, respectively. The boys in particular showed a tendency towards delayed bone age, whilst the girls were rather evenly distributed as regards early and late maturation. In the group with genetically determined dyslexia there was one boy, and in the group with no dyslectic heredity there were two boys showing cryptorchism. In the latter group there was, moreover, one boy who exhibited a malabsorption syndrome and short stature (Table 2).

*Psychosomatic symptoms.* Somatic manifestations of both a disturbed psychophysical balance and prolonged tension were frequent. The commonest symptoms were abdominal pain, fatigue, dysmenorrhoea and headache among the girls, headache, fatigue and abdominal pain among the boys. (Table )

TABLE 2 *Physical observations*

| | Group with known heredity | | | | Group without known heredity | | | |
|---|---|---|---|---|---|---|---|---|
| | Girls | % | Boys | % | Girls | | Boys | % |
| *Anamnestic data* | | | | | | | | |
| Protracted primary enuresis | 3 | 11 | 13 | 25 | 1 | 8 | 3 | 10 |
| Speech disturbances | 1 | 4 | 8 | 15 | 1 | 8 | 1 | 3 |
| Poor motoriety | 10 | 36 | 31 | 60 | – | – | 15 | 52 |
| *Physical development* | | | | | | | | |
| Delayed bone age | 3 | 11 | 11 | 31 | 2 | 15 | 11 | 38 |
| Late menarche | 3 | | | | 1 | | | |
| Total no. of cases | 5 | 18 | 11 | 21 | 2 | 15 | 11 | 38 |
| Advanced bone age | | 7 | 3 | 6 | 2 | 15 | 3 | 10 |
| Early menarche | 5 | | | | 2 | | | |
| Total no. of cases | 5 | 18 | 3 | 6 | 3 | 23 | 3 | 10 |
| *Neurological observations* | | | | | | | | |
| Pathological EEG total number | 8 | 29 | 29 | 56 | 7 | 54 | 13 | 45 |
| Clearly lesional | 1 | | 7 | | 3 | | 8 | |
| Lefthandedness | 4 | 14 | 5 | 10 | 1 | 8 | 5 | 17 |
| Speech disturbances | 1 | 4 | 11 | 21 | 2 | 15 | 4 | 14 |
| Enuresis | – | – | – | | – | – | 1 | |
| Immature motoriety | 13 | 46 | 28 | 54 | 5 | 38 | 8 | 21 |
| Tics | 1 | | 3 | | – | | 1 | |
| Slight spastic pareses | – | | 1 | | – | | 2 | |
| *Physical signs and symptoms* | | | | | | | | |
| Total no. of cases with functional physical symptoms | 17 | 36 | 30 | 58 | 9 | 69 | 14 | 48 |
| dysmenorrhoea | 6 | | | | 2 | | | |
| abdominal pain | 6 | | 5 | | 2 | | 4 | |
| fatigue | 5 | | 9 | | 5 | | 4 | |
| frequent diarrhoea | 2 | | 1 | | – | | 2 | |
| headache | 5 | | 10 | | 3 | | 3 | |
| obesity | 1 | | 2 | | 2 | | 1 | |

*Psychic and social observations*

*Psychic symptoms* In the majority of cases dyslexia was associated with disturbed emotional balance. There were only 6 cases in which no neurotic symptoms occurred. Restlessness, distractability, anxiety, sleep disturbances, depression, as well as lability and nailbiting were typical features. Lack of assurance led to selfassertion, aversion against school, school tension and in some cases to a "dullness" complex. (Table 3)

*Dyslexia and intelligence* Assessments of intelligence according to Wechsler Adult Intelligence Scale (WAIS) or Wechsler Intelligence Scale (WISC) for Children were performed in all cases except 19 in which previous tests or

TABLE 3 *Psychic and social observations*

| | Group with known heredity | | | | Group without known heredity | | | |
|---|---|---|---|---|---|---|---|---|
| | Girls | % | Boys | % | Girls | % | Boys | % |
| *Psychic symptoms* | | | | | | | | |
| Total no. of cases with neurotic symptoms | 27 | 96 | 49 | 94 | 12 | 92 | 37 | 92 |
| lack of assurance | 20 | 71 | 32 | 67 | 6 | 46 | 14 | 40 |
| aggression and self assertion | 5 | 18 | 11 | 21 | 2 | 15 | 7 | 4 |
| school tension | 12 | 46 | 17 | 33 | 2 | 15 | 13 | 45 |
| "dullness" complex | | 7 | 7 | 13 | 1 | | 2 | 7 |
| sleep disturbances | 8 | 29 | 17 | 33 | 4 | 31 | 13 | 45 |
| anxiety and restlessness | 10 | 36 | 16 | 31 | 2 | 15 | 11 | 38 |
| asocial behaviour and problems of adjustment | – | – | 6 | 1 | 1 | 8 | 4 | 14 |
| distractability | 6 | 21 | 21 | 40 | 3 | 23 | 12 | 42 |
| depression | 15 | 54 | 25 | 48 | 4 | 31 | 12 | 42 |
| school aversion | 6 | 1 | 8 | 15 | 1 | 8 | 2 | 7 |
| sexual problems | 1 | 4 | 1 | 2 | – | – | 1 | 3 |
| lability | 5 | 18 | 7 | 13 | 3 | 23 | 7 | 24 |
| nail biting | 7 | 25 | 9 | 1 | | 15 | 7 | 24 |
| *Intelligence* | | | | | | | | |
| IQ ≥ 110 | 8 | 32 | 13 | 30 | 2 | 20 | 7 | 24 |
| IQ 100–109 | 7 | | 18 | | 5 | | 9 | |
| IQ 90–99 | 6 | | 8 | | | | 8 | |
| IE under 89 | 3 | | 4 | | 1 | | 1 | |
| Difficulties relating to form | | 7 | 8 | 15 | 4 | 31 | 8 | 28 |
| Verbal part of test > 10 points lower than performance part | 5 | 18 | 12 | 23 | | 15 | 7 | 24 |
| Verbal part of test > 10 points higher than performance part | 1 | | 7 | | – | | 1 | |
| Irregular test profile | 14 | 58 | 33 | 8 | 7 | 35 | 14 | 50 |
| Y test | 4 | | 8 | | 3 | | 4 | |
| *Social conditions* | | | | | | | | |
| Divorce | 2 | | 7 | | 2 | | 4 | |
| Death of parent | 4 | | 2 | | 5 | | 1 | |
| Marital conflicts, insecurity | 3 | | 10 | | 6 | | 6 | |
| Total no. of cases with stressing home situation | 9 | 33 | 18 | 35 | 8 | 62 | 11 | 25 |
| Parental dissatisfaction and pressure | 4 | | 5 | | 3 | | 5 | |

definite evidence of high intelligence were available. Mostly a relatively high intelligence quotient was noted. (Table 3) In 9 cases only was the total intelligence quotient under 89, the lowest noted being 78.

The intelligence quotients obtained gave no adequate idea of the intellectual

TABLE 4.

| | Group with known heredity | | | | Group without known heredity | | | |
|---|---|---|---|---|---|---|---|---|
| | Girls | % | Boys | % | Girls | | Boys | % |
| *School problems* | | | | | | | | |
| *Delay in referral change of school and reading difficulties* | | | | | | | | |
| Delay in referral once | 11 | | 14 | | 5 | | 15 | |
| Delay in referral twice | 4 | | 11 | | 2 | | 1 | |
| Delay in referral three or more times | 2 | | 6 | | — | | 2 | |
| Total no. of pupils with delay in referral | 17 | 61 | 31 | 60 | 7 | 54 | 18 | 6[illegible] |
| Change of school | 3 | 11 | 16 | 31 | | 15 | 6 | 21 |
| Badly treated in peer group | 1 | 4 | 9 | 17 | 2 | 15 | 6 | 21 |
| Reading difficulties | 13 | 46 | 27 | 52 | 6 | 46 | 12 | 41 |
| Difficulties in arithmetic | | | 3 | | 1 | | 5 | |
| *Writing-mistakes* | | | | | | | | |
| Errors of form | 20 | 71 | 41 | 79 | 11 | 85 | 19 | 66 |
| Reversion and omission | 19 | 68 | 45 | 87 | 13 | 100 | 22 | 76 |
| Confusion of double/single consonant | 27 | 96 | 49 | 94 | 13 | 100 | 28 | 97 |
| Confusion of å/o | 4 | | 45 | | 11 | | 21 | |
| Confusion of [illegible]/å | 19 | | 39 | | 10 | | 19 | |
| Confusion of simple and compound words | 9 | | 9 | | 1 | | 4 | |

levels of these individuals. The test results were impaired by a very irregular test profile (a difference of ≥7 points between partial tests). It was also striking that there often was a marked difference between the quotients for the verbal and performance parts. In 22 cases, the majority of which belonged to the group without familial dyslexia, difficulties in the distinction of form were noted in the test.

*Social stress.* Often the social situation of these boys and girls was complicated by excessive expectations, disapproval and lack of understanding. Social stress due to broken homes, marital conflicts and insecurity was also a noteworthy feature (Table 3).

*School problems*

*The frequency of delay in referral* was as high as 60 per cent and many boys and girls had failed to be referred two three or even more times. There was no difference between those showing genetically determined dyslexia and the remainder in this respect. The situation was in some cases further complicated by frequent changes of school. Among the boys in particular such changes were often necessitated by delays in referral two successive years, or by problems of adjustment. The boys in particular were often badly treated in the peer group. (Table 4).

In 40–60 per cent of the present series

effective studies were further hindered by *reading problems* in the sense that the pupils in question were unable to read fluently: they made mistakes and often faltered at foreign words. Furthermore they read very slowly, which obviously prolonged the time required for the preparation of lessons. In extreme cases the contents of a text were not comprehended owing to the difficulty in reading. Sometimes the dyslexia caused problems in arithmetic because the meaning of textual tasks was not grasped.

The *writing-mistakes* made in Swedish were analysed on the basis of a large number of written tasks which had been done at school or outside it. The factors considered were 1) errors of form, e.g. b-d p-g (eidetic phenomena, according to Kirschoff [7]), 2) reversion and omission of letters, 3) mistakes in respect to double/single consonants, 4) confusion of e and ä and 5) of o and å, 6) inability to discriminate between simple and compound words. The most frequent problems were caused by single/double consonants, but errors of form and reversions were also very common. (Table 4)

*Dyslexia and the environment.* The reasons for contacting our clinic sometimes disclosed how the disturbance had been *interpreted. In regard to one-third of the* girls and one fourth of the boys with known familial dyslexia quite other reasons than difficulties in writing were indicated. Of those who had no family history of dyslexia about half were referred to the clinic on account of other causes, the main complaints being physical or neurotic symptoms although poor adjustment and vaguely described school problems also were indicated. In somewhat over *0 per cent of cases laziness, stupidity or carelessness were mentioned as the cause of the backwardness in writing. The expression most frequently used was carelessness, the next commonest being laziness and stupidity.

*Etiological factors in dyslexia*

The possible presence of a *hereditary predisposition to dyslexia* was analysed by asking the parents whether they or any near relatives had experienced serious difficulties in learning to write. In regard to 28 girls (68 per cent) and 52 boys (64 per cent) it was found that their parents or some near relatives had been backward writers. This trait was about equally often encountered on the maternal as on the paternal side. In 13 cases a hereditary predisposition occurred in the families of both parents. Writing difficulties among siblings were noted in about 27 per cent of cases, and equally often among the siblings of the girls as among those of the boys. In 43 cases the *hereditary predisposition was combined with other possible causes* of dyslexia. Brain damage was regarded as probable in 10 cases, general and neurogenous dysmaturity in *4 and diffuse encephalopathy in one case. In a further 7 cases etiological factors other than a hereditary tendency were suspected but uncertain. Among these 3 girls showed general developmental retardation, and the boys suffered probably from the effects of brain injury. (Table 5)

Thirteen girls (3_ per cent) and 9 boys (36 per cent) had *no family history* of dyslexia. In this group postlesional encephalopathy was observed in 3 girls and 10 boys. Apart from these cases, encephalopathy occurred *as a result of*

TABLE 5 *Etiological aspects*

| | Girls | % | Boys | % |
|---|---|---|---|---|
| Total no. with known heredity | 28 | 68 | 5[illegible] | 64 |
| mother dyslectic | 12 | | 16 | |
| father dyslectic | 11 | | 22 | |
| both parents dyslectic | 4 | | 9 | |
| only siblings dyslectic | 1 | | 3 | |
| total no. with dyslectic siblings | 11 | 27 | 22 | 27 |
| Total no. with known heredity in association with other possible causes, e.g. | 12 | 29 | 31 | 38 |
| brain damage | 2 | | 8 | |
| diffuse encephalopathy | 1 | | – | |
| general neurogenous dysmaturity | 5 | | 19 | |
| uncertain cases | 4 | | 3 | |
| Total no. without known heredity | 13 | 32 | 29 | 36 |
| postlesional cases | 3 | 23 | 10 | 34 |
| encephalopathy | 1 | | 1 | |
| general neurogenous dysmaturity | 1 | | 5 | |
| cases with obscure etiology | 8 | | 13 | |

toxoplasmosis in one girl and in association with a malabsorption syndrome and short stature in one boy. Neurogenous dysmaturity was present in 6 cases. In 21 cases, i.e. 8 girls and 13 boys, no clear etiology could be elicited. Among these there were 5 girls and 5 boys who were suspected of brain damage (Table 5).

Summing up it may be stated that a family history of dyslexia could be elicited in about two-thirds of the present cases. Central nervous dysmaturity was observed in 6 girls (15 per cent) and 24 boys (30 per cent). Postlesional encephalopathy was obviously present in 5 girls (12 per cent) and 18 boys (22 per cent) and probably in a further 5 girls and 8 boys. Thus, the probable frequency of significant brain damage was 24 per cent among the girls and 32 per cent among the boys. A hereditary predisposition seemed to be the only etiological factor in about one third of the present series, whilst another third showed a hereditary tendency in association with other factors, e.g. encephalopathy or general neurogenous dysmaturity; one third had no family history of dyslexia. In this group postlesional encephalopathy constituted the main etiological factor.

## Discussion

In a previous study [*] was found that among adolescents who made more writing mistakes than the average of their school class the writing difficulties partly seemed to be associated with the general distractability of puberty and with particular psychic conflict mechanisms. In regard to the series, however, it should be noted that cases of *pseudodyslexia* due to primary neurotic disturbances, to a poor intellect or insufficient linguistic knowledge had been omitted.

The boys were twice as numerous as the girls in the material. This does not warrant any conclusion regarding the possible existence of a sex linked hereditary trait [6], since the present series must be regarded as selected and may in part be attributable to the fact that success in school is regarded as more important for boys and to a poorer compensatory capacity of the boys.

These boys and girls experienced early difficulties in secondary school, particularly in Swedish (their native language) but also in foreign languages. Considering the ages of these individuals it must be stated, however, that many came for investigation so late that a variety of

secondary problems had already developed in respect to both the school and home situation and their psychic health.

The *psychic situation* of these boys and girls was depressing. Lack of understanding and pressure both in the home and in the school caused a further stress at the same time as their own shortcomings and the experience of inadequacy and incapacity created a *serious conflict* situation. Social insecurity accentuated the handicap and augmented the problems and the secondary neurotic symptoms. In particular the possibilities of compensation were diminished by broken homes and conflicts in the home, sometimes consisting of conflicts between the parents due to the school and writing problems of the child or to a sense of guilt and insufficiency because of the latter.

The protracted tension and the conflict situation were accompanied by a variety of functional somatic symptoms. We found in a previous study an increased frequency of dyslexia among obese adolescents [3]. But also the constitutional weakness must be taken into account for the development of these symptoms.

*Lack of assurance and anxiety were* conspicuous features, but problems of adjustment accompanied by tendencies to self-assertion and even to asocial behaviour were also noted. Frequently aggression developed in response to unfair treatment. More often, however, falling self-esteem, depression and a dullness complex resulted from the repeated failures and disappointments and the experience *of not* being able to meet one's own expectations or the expectations of others.

The school problems and writing difficulties were not due to a low intellectual level in any of the present cases. The *intelligence* quotients obtained represented *minimum performances*, since previous shortcomings and secondary neuroses in combination with tension on account of the test situation produced uncertain and uneven results. The performance tasks *often seemed to be stimulating. Obviously* arithmetic tasks, in particular, caused fear and aversion. The results in the performance part were negatively influenced by various factors such as lack of self-reliance, lability and a poor capacity for grasping and planning the total situation. Sometimes nervous or slow hand motoricity, occasionally combined with pedantic meticulousness, constituted a drawback. *In many cases these traits* were compensated by a mobile and rapid intellect. Although these boys and girls were often able to express themselves well and logically, the results on the verbal part were impaired by poor information due to very limited reading. Considering the intellectual and social levels of the test subjects this mostly implied a slightly lowered *IQ* for the verbal part of the test.

The WAIS and WISC results exhibited no definite test profile which would have enabled distinction of the different subgroups of dyslexia, but a general irregularity was obvious.

In the groups showing damage and encephalopathy, too, *IQ* was, contrary to the usual finding in adults with organic brain injuries [0], higher in the performance part than in the verbal part.

The *school situation* was often a tragedy. Perpetual shortcomings made life

disagreeable and cast a gloom over the future The backwardness in reading meant prolonged work and necessitated private lessons, and thus the time available for leisure and hobbies was curtailed. Nagging and urging for better results and more work occurred in the home and in the school, too. The consequences was a "dullness" complex, school tension and aversion against school. The difficulties led to an averseness to all reading and thus to a limitation of the reading of literature and the acquiring of general knowledge School was especially a trouble in those cases in which the pupil was incapable of learning his lessons on his own.

The type and composition of the *writing-mistakes* were analysed on the basis of written specimens from various ages. In the group with familial dyslexia, in particular a change for the better occurred with increasing maturity of the central nervous system, so that in the last stage of the dyslectic disturbance the errors made in writing were no longer "typical" It seemed to be of importance for the assessment to recapitulate the writing mistakes made in earlier years. Temporary changes in the writing performances were also observed. Although the frequency of mistakes was sometimes low under optimal circumstances it could obviously be increased by transient impairment of the alertness due to infection or fatigue or nervous distractability A noteworthy factor was the presence of secondary neurosis, it being obvious that lack of assurance and doubts concerning one's writing capacity increased the rate of errors.

The backwardness in writing had often been misinterpreted in particular in the group without known familial dyslexia But in the group with genetically determined dyslexia too the parents were often guilty of misunderstanding the situation and of a prejudiced attitude The attitude of the teachers could not be analysed, but from comments in the writing books perused the conclusion could be drawn that in too many cases their knowledge and understanding had been insufficient.

In respect to the *etiological factors* an attempt was made to clarify the hereditary circumstances by questioning the parents. The information obtained could not be checked. The advanced school education that was common in these families seemed however to warrant a certain degree of reliability Among the obvious causes a hereditary tendency was predominant. It was equally often derived from the maternal as from the paternal side The penetration of this trait seemed to be very high since dyslexia was of common occurrence among the siblings of the present subjects.

A hereditary dyslectic predisposition often occurred in combination with other factors as brain damage which could be of etiological significance This observation argues in favour of the occurrence of a specific punctum minoris resistentiae in the central nervous system of individuals showing genetically determined dyslexia In other cases the herditary predisposition was combined with neurogenous dysmaturity or with a retardation of the whole development

The third etiological main group showed no hereditary predisposition to dyslexia. In this group postlesional encephalopathies constituted the most important cause

Furthermore dysmaturity was noted in some cases. This finding seems to argue in favour of the view that a particular disturbance of physical maturation and development associated with dyslexia exists in which no hereditary dyslectic predisposition is involved. In many cases the etiology remained obscure however

A retarded development in combination with neurogenous dysmaturity was more often encountered in the boys than in the girls. It seems possible that a sex linked *genetical trait is involved. In our study* of adolescents showing delayed physical maturation [4] a correlation with dyslexia was disclosed If the above mentioned general developmental disturbance and the overrepresentation of boys with postlesional encephalopathies is taken into account the difference between boys and girls in regard to the frequency of dyslexia is slight. These observations may indicate that another form of hereditary dyslexia occurs which is not sex linked.

The present results constitute evidence in favour of a causal relationship between dyslexia and a functional disturbance of the central nervous system This conclusion *is strongly corroborated by frequent* histories of protracted primary enuresis motoric handicaps and disturbed motoric development and by the occurrence of speech difficulties Furthermore, in many cases the encephalogram showed deviations from the normal. The changes seemed to be due sometimes to diffuse lesions, sometimes to dysmaturity of the central nervous system Changes in motoric skill were often involved. These changes were probably associated with poor differentiation and poor maturation of the central nervous system or with diffuse injuries. In the evaluation of the motoric functions, the possible effect of nervous tension and other disturbing influences must be considered

Moreover the hypothesis that dyslexia has a physical origin is supported by the observations relating to the physical development, in particular the high frequency of deviations in bone age It should be recalled that delayed bone age was particularly common among the boys. In a study of normal teenagers, *which is in process at our clinic and in* which the same methods and criteria are applied as in the present investigation advanced bone age was found to be very infrequent among the girls, whilst 0 per cent of the boys exhibit this feature In the present series an advanced bone age whas somewhat more frequent among the girls than among the boys. In the normal series delayed bone age was noted in 2 per cent of the girls and in 14 per cent of the boys. Against the background of the results in the normal series it appears that the frequency of delayed bone age in particular is increased in both sexes in the present series *The results corroborate the* conclusions drawn regarding a correlation between retarded maturation and dyslexia but they also seem to indicate that diffuse brain injuries may be involved when developmental deviations occur in the form of either advanced or delayed maturation.

The dyslexia was often both a personal and a family tragedy It reduced the chances of further education, undermined the belief in a happy future and constituted a handicap that prevented self realization. The present study showed

that not only parents are badly in need of information on this point, teachers, too often seem to be very poorly informed as regards dyslexia. In the schools special provision for these pupils ought to be available in order to counteract their handicap. All this is urgently needed if they are to make a future for themselves and avoid persistent neurosis and frustration.


## Summary

A study of dyslexia in teenagers showed that a complex problem is involved. In many cases writing and reading difficulties were accompanied by difficulties in arithmetic. Since individuals with a low intellectual level or with visual or hearing defects had been omitted from the series, the etiological factors that remained to be considered were either hereditary or lesional or a combination of these. In some cases dyslexia was associated with a general disturbance of the physical development involving neurogenous dysmaturity and delayed bone age. This condition seemed to occur among the boys and is probably sex linked. The dyslexia caused difficulties in the school and often made it impossible for the individual to make the best of his intellectual abilities. The situation was complicated by family problems and external pressure. It was found that parents and teachers are not sufficiently informed regarding the nature of dyslexia and therefore conflicts and tension develop. The result is that psychosomatic and neurotic symptoms as well as problems of adjustment occur in abundance among the teenagers suffering from dyslexia.



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# An Epidemic of Otitis in Newborns due to Infection with *Pseudomonas Aeruginosa*

*by* LARS VICTORIN

Otitis of the external or media type is rare in the newborn period and in general is not specifically mentioned in current textbooks of pediatrics or ear-nose-throat diseases with some exception [17 20] It has been reported as occurring mainly in connection with prematurity malformations, chronic or other debilitating conditions [15 20].

Iatrogenic infections with *pseudomonas aeruginosa* (P A ) have been reported in recent literature [3 9 11] and the incidence of such infections is probably increasing The widespread use of technical apparatus, which incorporates water or humidified gases, with associated difficulties in sterilization, are considered largely responsible for this increase [3 6]

The present report includes a series of newborns with infections of the external auditory canal and middle ear and points at one of the potential hazards of routinely bathing newborn babies in hospitals

## Material and procedures

During a period of } months 7 cases of otitis due to infection with P.A. were noted. The total number f births in the hospital in question is around 4600/year All babies were delivered vaginally were full term and in good general condition. Details of the cases are given in T ble 1

Routine treatment of newborns in the delivery ward at this time included bathing nd gentle cleaning in water soon after birth mainly for aesthetical reasons. After drying the baby was placed in a bed lying on the right side in order to f ce the mother during the two hours they remained in the delivery room after birth. Thereafter mother and child were transported t one of the maternity wards where they stayed for 5– days.

In all cases suppuration from the left auditory canal became obvious on the 4–5 day of life except in case I where for reasons unknown, the infection was in the right ear Examination by an ear-nose-throat specialist revealed external otitis in all patients, pronounced myringitis in 6 and proven middle ear infection in one. Two cases were called "probable middle ear infection but were not subjected to myringotomy Bacterial examination was performed on the suppurat and on external uditory canal swabs in every baby and lso on the material btained from myringotomy in case nr 3. The bacteria cultivated was without exception P A. with resistance patterns as given in Table 1

Apart from the local infection the babies were clinically normal and laboratory data, when tested, were normal with respect t whit blood count, sedimentation rate haemoglobin and reticulocyte count

TABLE 1 *Clinical and laboratory data of 7 newborn infants with pseudomonas aeruginosa otitis*

| N | Birth date | Name | Birth weight g | Otit. Ext. (Side) | Myringitis | Otit. Media | Myringotomy | Treatment | Local cleansing | Local tetracycline-steroid ointment | Resistance pattern Pseudom. ear: Bath | Penicillin | Tetracycline | Chloramphenicol | Strepto mycin | Ampicillin | Polymyxin | Day of first sign |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 66 04 12 | S | 3400 | right | + | – | – | Ampicillin | + | – | 0 | 0 | 1 | 0 | 3 | 0 | 7 | 5 |
| | | | | | | | | | | | 3 | 0 | 3 | 1 | 3 | 0 | 3 | |
| | 66 04 26 | H | 2500 | left | + | – | – | Penicillin | + | + | 0 | 0 | 1 | 0 | 3 | 0 | 3 | 5 |
| | | | | | | | | | | | 0 | 0 | 0 | 0 | 3 | 0 | 3 | |
| 3 | 66 05 06 | K | 3510 | left | + | + | + | Penicillin | + | – | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 6 |
| | | | | | | | | | | | 3 | 0 | 0 | 0 | 3 | 0 | 3 | |
| 4 | 66 05 17 | K | 3150 | left | – | – | – | – | + | + | 3 | 0 | 1 | 0 | 3 | 0 | 3 | 5 |
| 5 | 66 05 22 | L | 3000 | left | + | (+) | – | Penicillin | + | | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 4 |
| | | | | | | | | | | | 3 | 0 | 3 | 0 | 2 | 0 | 3 | |
| 6 | 66 05 29 | K | 3210 | left | + | – | – | Penicillin | + | – | 3 | 0 | | 0 | 2 | 0 | 3 | 4 |
| 7 | 66 06 27 | H | 3400 | left | + | (+) | – | Ampicillin | + | – | 3 | 0 | 1 | 3 | 3 | 0 | 3 | 4 |

( ) = suspected otitis media not verified by myringotomy. Resistance pattern 0 not sensitive, 3 maximal sensitivity.

Treatment consisted of initial local cleansing and in two cases application of tetracycline-steroid ointment. Before bacteriological diagnosis was known 4 babies received oral penicillin, 2 others oral ampicillin. On re-examination 8 to 14 days after the first appearance of signs all cases had normalized.

A thorough bacteriological investigation of localities and of personnel revealed growth of P. A. in the bath water used for the newborns in the delivery rooms and also in the outlets from the water bassinets. These were being cleaned after each baby with a liquid detergent practically without any antibacterial effect. Bacterial cultivation from previously unopened bottles of this product was positive in regard to P. A.

After switching over to cleaning both babies and bath-bassinets with another agent (hexachlorophene) no new cases of otitis in newborns have been seen over the past 8 months.

## Discussion

In the literature on hospital infections with P. A. the importance of contaminated water or mist as carriers of the bacteria is repeatedly stressed [9, 1, 22, 4]. The most favourable condition for the growth of this bacteria is with still water at between room and body temperature [13].

Epidemiological studies on P. A. infections in wards for newborns show that systemic infection is comparatively rare, comprising 3–4 % of all cases in which P. A. are isolated, but also that when complicating the picture it carries a very poor prognosis [18]. It is estimated that around 20 % of all cases of sepsis in the newborn period are due to P. A. infection [8]. A common finding is that

laboratory data do not give much positive information regarding this diagnosis however possibly a shift to the left in the white blood picture and rarely thrombocytopenia is seen [15].

In the light of what is thus known about the characteristic behaviour of P A and the methods used in taking care of newborns in the ward in question a very probable sequence of events could be arrived at The bath bassinets were cleaned with a contaminated detergent that was not completely washed away and thus to some extent dispenced in the bath water for the next baby Water entered the external auditory canals during bathing, and as the baby was placed on his right side spontaneous drainage of that ear took place while water remained in the left auditory canal. The water repellent covering usually prevents intimate contact between the water and the surface cells but with time the oily secretions may be dissolved making it possible for clinical infection to supervene [23]. This was the case after a rather constant incubation period of 4–5 days in all our patients.

Two cases also had suspected and one proven middle ear infection with P A. It is unlikely that this spread of the infection had taken place directly from the external auditory canal as judged from the current opinions that otitis media is almost invariably mediated through the Eustachian tube or hematogenously If this is true also with the newborn is not known. If so a probable explanation would be that apart from the water in the external auditory canal some also entered the upper airways giving rise to an ascending middle ear infection.

Local cleansing and, when applied tetracycline-steroid ointment has probably been of benefit in the cases reported, whereas, judging from the resistance patterns, systemic antibiotics were of little or no value In severe infections with P A. which was not the case here, colistin or polymyxin B sulfate are recommended as the drugs of choice [15].

The whole question of bathing newborns routinely for cleansing is controversial. The most common opinion seems to be that there is no real need for routine baths but instead to rely on gentle sponging preferably with added hexachlorophene [4, 7 10 14 16 19 21 25]. Other authors put forward the idea that delay in bathing and leaving the vernix caseosa in skin folds sometimes favours skin infections. However the importance of always drying out the auditory canals is specifically stressed [5 16]

'Standards and recommendations for hospital care of the newborn infant edition 1954 [1] gives the opinion that it is essential to the protection of the infant to avoid water or oil baths during the hospital stay while in edition 1964 [2] it is said that hexachlorophene bathing probably reduces the rate of skin and nasal colonization by staphylococci and possibly lowers the incidence of skin infections in the newborn infant Other bacteria and sites of infection are not mentioned Any pronounced effect in prophylaxis against P A. infection is not to be expected from hexachlorophene and this product was chosen for routine use mainly for its effect on the quantitatively dominating staphylococci infections. Infections due to P A. are best avoided by general hygienic measures with special

regard to moisture or water in contact with the newborn.

## Conclusion

With the epidemic described as a background it seems reasonable to conclude that bathing the newborn baby in the delivery room or maternity ward is not necessary and could carry certain risks. If performed it should take place with an antibacterial agent added to the water. Special care should be taken not to leave any water in the auditory canals and regular bacteriological checking of bath bassinets should be a rule.


## Summary

An epidemic of pseudomonas otitis in newborns due to infected bath water is described. The question of the necessity of bathing newborn babies in delivery rooms or maternity wards is briefly discussed.



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# Suicidal Attempts made by Psychotic Children and Adolescents

by ULF OTTO

In an earlier paper Bergstrand & Otto [1] have reported on a material covering 1727 Swedish children and adolescents under 21 years of age who in the years 1955–1959 were treated because of suicidal attempts in hospitals and other institutions in Sweden. In that survey an account was given of a number of external conditions in connection with the suicidal act. On the basis of the same material Otto has made an intensified analysis of psychiatric states of illness and personality variables [11] the existence of a presuicidal syndrome [15], and suicidal attempts made by conscripts [10], schoolchildren [13], pregnant women [14] and children under 14 years [1 ]. The aim of the present survey based on the same material, is to study more thoroughly children and adolescents under 21 years, who in the examination following on the suicidal attempt were considered to suffer from a psychotic state of illness.

## Materials and Methods

The principle followed in the collection of the material, and the method of selection has been explained previously [1] For the five year period 1955–1959 the case notes were collected and studied on patients under 1 years who had been treated because of suicidal attempts in all hospitals in Sweden to which cases of this nature could supposedly be referred. This implies that a total of 471 hospitals and other institutions were approached, 465 (98.8 %) of which forwarded case notes, covering 1727 cases in all. The treatment of such material must be uneven and therefore only such data as sex, age, and the character of the suicidal ttempt have been recorded for the entire material. Approximately 1/3 of the cases had undergone psychiatric exploration. The distribution by sex and age of this portion show a statistically satisfactory accordance with the entire material. On those more thoroughly penetrated cases more detailed data and psychological facts have been brought out, such as heredity conditions of growth and environment past illnesses, personality as well as mental and somatic morbid conditions. The uneven manipulation of the cases and the journals in different hospitals and by different physicians is naturally weakness in a material of this nature but is counterbalanced by the fact that it covers the entire country and that it is not limited to a restricted lientele derived from given district, hospital department, or hospital.

The various diagnoses should be interpreted in their widest sense due to the difficulty in defining psychiatric states of illness and personality variables and with view to the umber of xaminers.

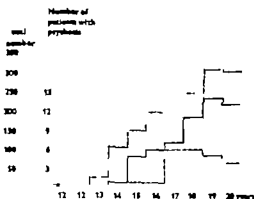


Fig 1 Distribution by ge of children and adolescent having ttempted suicide. ——— schizophrenia; – – – manic-depressive psychosis; — — rest of the material.

Statistical analysis was based on the $\chi^2$ test The degree of probability was denoted as follows:

Almost significant $1\% < p < 5\%$
Significant $0.1\% < p < 1\%$
Highly significant $p < 0.1\%$

*Here, p signifies the probability of the* difference having been due to chance.

## Results

An account of the distribution of ill nesses among those having undergone psychiatric exploration is given in Table 1 Neurotic states dominate, 53 0 % *followed by psychotic states*, 16.5 %, of which schizophrenia constitutes 11.8 % and manic-depressive psychosis 4 7 %

In schizophrenia boys predominate (Table 2) as compared to girls, 19.2 and 9.2 % respectively (**) whereas in manic-depressive psychosis no difference exists between the sexes, boys 5 6 % and girls 4.5 %.

In schizophrenia as in *manic-depressive* psychosis the distribution according to age corresponds in the main to that of the remaining material (Fig 1) The youngest child is a 14-year-old boy

I 14; 9-year-old boy 3/4 who attends grammar-school (realskola) During his years of growth he has had a mother fixation, been softhearted, sensitive, shy and pedantic. At school he has been easily irritated and often involved in conflict with his environment. At the age of 12 he has expressed weariness of life and has had periods of vagrancy H has gradually become increasingly irritable He attempts suicide by endeavouring to shoot himself with a rifle In a letter found he tells about his dream girl and the imagined experiences he has had in a dream-paradise. He is unhappy at home and at school. Psychiatric opinion: Schizophrenic syndrome with paranoid delusions in a well-gifted, asthenic personality

TABLE 1 *Distribution of psychiatric states of illness*

| Psychiatric states of illness | No. | % |
|---|---|---|
| Neurotic-depressive reaction | 144 | 29.8 |
| Neurosis | 112 | 23.. |
| Psychosis | | |
| Schizophrenia | 57 | 11.8 |
| Manic-depressive psychosis | 23 | 4.7 |
| Psychopathy | 64 | 13 2 |
| Adolescent adjustment reaction | 52 | 10.7 |
| Brain damage | 32 | 6.6 |
| Total | 484 | 100.0 |

TABLE 2 *Distribution of psychotic states in boys and girls*

| Psychotic stat | N | Boys No. | Boys % | Girls No. | Girls % | Diff. ♂/♀ |
|---|---|---|---|---|---|---|
| Schizophrenia | 57 | 24 | 19.2 | 33 | 9.2 | – |
| Man. depr ps. | 23 | 7 | 5.6 | 16 | 4.5 | – |
| Total (psychoses) | 80 | 31 | 24.8 | 49 | 13 7 | – |

TABLE 3. *Distribution by social groups*

| Social group | Schizo-phrenia No. | % | Man. depr psychosis No. | % | Rest of material No. | % | Diff. psych. material |
|---|---|---|---|---|---|---|---|
| I | 7 | 15.6 | 3 | 15.8 | 85 | 9.5 | |
| II | 4 | 8.9 | 3 | 15.8 | 64 | 7.3 | |
| III | 34 | 75.6 | 13 | 68.4 | 747 | 83.2 | |
| Total | 45 | 100.0 | 19 | 100.0 | 896 | 100.0 | |

The distribution according to social groups, always difficult to determine in view of definition difficulties, have in cases of schizophrenia and manic-depressive psychosis been compared with the remaining material (Table 3) Social groups I and II are represented by 24.4% in schizophrenia, and 31.6% in manic depressive psychosis, in II psychoses by 26.6%, while they represent 16.8% in the remaining material. The difference between the psychoses and the remaining material is almost significant (*)

Registered causes of the suicidal act have been reported in Table 4 In the material exclusive of the psychoses exogenous moments in the form of love problems, 41.3% home and parental problems, 34.5%, and school problems, 6.6%, are present to a considerably greater extent than in schizophrenia, where the illness as such has been indicated as the cause in 77._% and in manic-depressive psychosis in 87.0%. The difference between psychoses and the remaining material is highly significant (***) Description of the mental state in schizophrenia during which the suicide was attempted has been given in 45 cases. In 25 cases, 55.6%, a depressive reaction has dominated the picture and been judged an essential factor for the provocation of the suicidal attempt In the remaining 20 cases, 44.4%, more distinct schizophrenic symptoms dominate (hallucinations, impertitive acts, influence delusions, etc.)

Social group I corresponds to the high income class.

The ingestion of tablets represents a smaller part in schizophrenia than in the entire material 71.9 (41) and 87.0% (1441) respectively The difference is highly significant (**) Passive methods (narcotic drugs and gas poisoning) constitute in the material exclusive of psychoses 89.7% (1471) compared with schizophrenia, 73.7% (42) (***) and with

TABLE 4. *Reasons for suicidal attempts*

| Reasons | Schizophrenia No. | % | Man. depr psych. N | % | Rest of material N | % | Diff. psych. material |
|---|---|---|---|---|---|---|---|
| Love problems | 10 | 17.5 | 2 | 8.7 | 380 | 41.3 | |
| Home and parental problems | 2 | 3.5 | 1 | 4.3 | 316 | 34.5 | |
| School problems | 1 | 1.8 | – | – | 61 | 6.6 | |
| Mental illness | 44 | 77.[illegible] | 20 | 87.0 | 111 | 12.1 | *** |
| Military service | – | – | – | – | 17 | 1.8 | |
| Pregnancy | – | – | – | | 34 | 3.7 | |
| Total | 57 | 100.0 | 23 | 100.0 | 921 | 100.0 | |



Fig 2. Frequency of behaviour changes during the three months preceding suicidal attempts in different conditions of psychiatric illness.

manic-depressive psychoses, 67 0 % (20) There is thus a trend towards higher frequency of active methods in schizophrenia than in other states of illness.

The duration of the schizophrenic state *has been recorded in 42 cases. Of these 25* 59 6 %, have had the illness a year or less, while 17 40 4 %, have had it for a longer period, the longest period registered being six years.

After the suicidal attempt 46 of the schizophrenic, 80 7 %, and 17 of the manic-depressive psychoses, 73 9 %, were conveyed to mental hospital or psychiatric clinic The corresponding figure for the remaining material is 25 7 % (312 of 1269 registered) The difference between the resp. psychotic states and the remaining material is highly significant (***)

A changed behaviour during three months preceding the suicidal attempt has been found to be most common in psychoses, 50.0 % (Fig. 2) Among these the change occurs slightly less frequently in schizophrenia 47 4 %, than in manic-depressive psychosis, 56 6 %. The difference between these forms of psychosis is not significant. Both in schizophrenia and in manic-depressive psychosis the changes are mainly characterized by depressive symptoms, 42.4 and 72.2 % respectively followed by neurotic symptoms, 27.3 and 22.2% respectively (Table 5).

Depressive tendencies expressed in the diagnosis, are present in 167 cases, representing 34.5 % of the 484 cases, where psychiatric diagnoses were registered. Of these 144 86.2 %, are diagnosed as *reactio neurotica-depressiva* that means a psychogenic condition and 23 13.8 %, as manic-depressive psychosis, presumably endogenously conditioned states.

As regards methodology in respect to the suicidal attempt too tangible differ

TABLE 5 *Different kinds of behaviour change preceding suicidal attempts in schizophrenia and manic-depressive psychosis.*

| Type of change | Schizophrenia No. | Schizophrenia % | Manic depr psychosis No. | Manic depr psychosis % |
|---|---|---|---|---|
| Changes in respect t social behaviour | – | – | 1 | 5 6 |
| Extrovert changes of type increased irritability aggressiveness, unstable affectivity | 2 | 6 1 | – | – |
| Purely neurotic symptoms such as anxiety sleep disturbances, headache tics, etc. | 9 | 27.3 | 4 | 22.2 |
| Depressive symptoms | 14 | 42 4 | 13 | 72.2 |
| Psychotic symptoms such as hallucinations, delusions of influence nd persecution | 8 | 24.2 | – | – |
| Total | 33 | 100.0 | 18 | 100 0 |

ences exist between endogenous and psychogenic depressions. In the former active methods are used in 13 0 %, and in the latter in 1..6 %. Changed behaviour during three months preceding the suicidal attempt has been registered in 60.6 % (13) of manic-depressive psychosis and in 31.9 % (4..) of neurotic depressions. No statistically significant difference exists in this respect. In all instances the changes had a depressive orientation. A highly significant difference exists on the other hand between psychogenic and endogenous depressions as regards the cause of the attempted suicide (** ). In the former love problems predominate 43 0 %, and home and parental problems 31.9 %, while in the latter the mental illness per se predominates with 87 0 %.

## Discussion

Our study of literature of current interest shows, partly that the frequency of psychotic states in connection with suicidal acts varies substantially in different materials, partly that the problem has attracted scant attention as far as children and adolescents are concerned. This is presumably due to the difficulty in defining the concept of psychosis, especially in respect to younger ages. Kanner (6) has emphasized that fully developed mental illness in the form of schizophrenia and endogenous depression only account for a small part of children suicides. In the material analysed here consisting of children and adolescents under 1 years who have been treated in connection with suicidal attempts during the years 1933–1939 the psychotic states likewise represent a minor part 16 5 in all, of which schizophrenia corresponds to 2/3 (11.8 %) and manic-depressive psychosis to 1/3 (4.7 .)

Among the psychotic states schizophrenia is generally considered predominant [* 7 8] Ettinger & Flordh [3] and Stengel & Cook (10) find on the other hand that endogenous states of depression dominate. We have found that among children and adolescents schizophrenia is dominating among the psychoses.

In the entire material girls constitute 80 % and boys 20 %. In psychotic states the boys represent a larger part in manic-depressive psychosis they constitute 30 4 % and the girls 69 6 %, in schizophrenia 42 1 %, and the girls 57.9 .

The distribution according to social groups shows that in psychoses social groups I and II represent a larger part 29 6 %, than in the remaining material, 16.5 %, where there is a greater preponderance of social group III. The difference is almost significant and difficult to explain as is always the case where endogenously conditioned states and environmental conditions must be compared and the connection between them elucidated.

The cause of the suicidal act is often seemingly trivial and has for the entire material been love problems, 39 ., home and parental problems 32 1 ., school problem 0 * pregnancy 3 4 military service 1 . and finally mental illness 1 . Mental illness ha in schizophrenia been recorded as the cause in ** and in manic-depressive psychosis in 87 0 In these no external moment provoking the suicidal attempt has been mentioned as is the case in other st tes. The reasons for this may be several, presumably the investigator has

not penetrated more deeply the world of ideas and motives of the seriously mentally ill patient in the same manner as is done in neurotic and other states, where the provoking moment has presumably come to the fore more directly in conversations with the patient. Reychard & Tillman [17] have also precisely underlined the lack of motive as a characteristic factor for the suicidal act In schizophrenia the cause is undoubtedly more deep-seated such as extreme anguish and tension, hallucinations, compulsory feelings, namely pictures that are part of the symptomatology for the illness concerned and have been considered *per se* as the provoking moment. Schneider [18] has carried the reasoning further as he is of the opinion that suicide at the onset of schizophrenia may not be directly attributable to the psychosis, but may rather be the reaction of the still intact personality to the menace to the entire personality through the down breaking psychosis

Farberow *et al* [7] have emphasized that the suicidal act is provoked by a need of liberation from tension in too strenuous a life situation, or a situation where there is insufficient possibility of finding a suitable issue or solution. A corresponding deeper penetration can naturally not be undertaken on the basis of the present material. It is, however evident that in psychosis external tangible causes of the suicidal attempt are considerably less frequent than in non-psychotic states.

Reychard & Tillman [17] have pointed out that especially the acute onset of the illness is often preceded by extreme anguish and tension, not infrequently combined with depression. We have found that in 55 6% of the schizophrenics depression has dominated the picture whereas for the remaining 44.4% distinctly schizophrenic symptoms have dominated. The depressive feature is thus of essential importance not only in depressive states, but also in schizophrenia.

Example of a schizophrenic process with a depressive picture where the provoking moment is love problem.

2 20-year old unskilled woman worker whose parents are alcoholics and have divorced shortly before the occurrence of the event in question. The maternal grandfather has committed suicide. Miserable and strenuous conditions of growth. The patient has earlier been treated in a psychiatric clinic Diagnosis Schizophrenia. After discharge she has lived in her fiancé's paternal home. In connection with conflict with fiancé the patient is depressed, calls her father on the phone and says that she intends to commit suicide since everything is hopeless and boring" She ingests an unknown number of chlorpromazine tablet and is brought to hospital, where a depressively tainted picture of a schizophrenic with distinct hallucinations and delusions is observed.

Example of a schizophrenic state of illness with a strongly depressive feature in the picture

3. 40-year old woman, storehouse attendant whose father paternal uncle and paternal grandfather are alcoholics. Only child. Always shy and sensitive A few years before the event in question the patient has grown increasingly taciturn, says that she hears voices and believes that the environment is spying on her In connection with the parents divorce she becomes deeply depressed and ingests about twenty five chlorpromazine tablets. At the arrival at the hospital emerges, in addition to pronounced depression, a clear schizophrenic picture with paranoid delusions.

slight incoherence and auditive hallucinations. Patient is conveyed t mental hospital.

Among children and adolescents the ingestion of tablets is the dominating method in suicidal attempts, 87.9 %, if psychoses are exempted. In schizophrenia the frequency is lower 71.9 %. Active methods are used to a greater extent in schizophrenia than in the entire material in all states passive methods are predominant, however These observations are confirmed by Stengel [19] who points out that nonpsychotic patients use poisons three and a half times more often than psychotic patients. Between manic-depressive psychosis and the remaining material no difference exists in respect to the frequency of tablet ingestion, 82.7 and 87.9 % respectively. In the initial stage of the illness or of an episode of schizophrenia the suicidal risk has generally been considered most acute [4 16]. In the cases where it has been possible to judge when during the period of illness the suicide was attempted, we find that the dominating number has occurred during the first year 69.6 %, which confirms these impressions.

The schizophrenic have in 90 % and the manic-depressive psychoses in 74.0 % been conveyed to mental hospital or psychiatric clinic after the suicidal attempt whereas this has occurred but in 4.6 of the remaining material. The observation can undoubtedly be explained on the basis of the special haracter of the latter states.

The possibility of predicting a suicidal act ha been the object of several investigation later also on the basis of the present material [15]. The highest frequency of behaviour changes during three months preceding the suicidal attempt has been registered in schizophrenia (4 4 %) and in manic-depressive psychosis (56.5 %) namely about half of the psychoses. For the other conditions of morbid mental states the variation frequency is 30–40 per cent. The lowest frequency is found in the early or primary character disorder (23.5 ) Both in schizophrenia and in manic-depressive psychosis depressive symptoms dominate during the presuicidal phase 4 4 and 72.2 % respectively with tiredness lack of energy indolence apathy and depression. In schizophrenia they are followed by symptoms of a neurotic character such as anguish, restlessness anxiety sleep difficulties headache nailbiting thumbsucking tics, etc (27.3 %) and in but a mere fourth ( 4.2 %) by symptoms considered typical of schizophrenia namely hallucinations depersonalization phenomena, etc. In manic-depressive psychosis the largest group but one (22.  ) likewise consists of symptoms of a neurotic character This is in keeping with the indication of Motto & Greene [8] that signs of depression, on a neurotic psychoti and a normal basis should be especially observed. Kaelberg [6] found likewise that the majority of 56 Danish children and adolescent whom he had examined had shown depressi tendencies before the suicidal attempt.

## Summary

After a re ie of earlier investigations made by the  rt concerning suicidal attempt in childhood and adolescence and fter discussion of the methodology

to be used in surveys relating to suicidal acts an analysis of a group of children and adolescents having attempted suicide in a psychotic state is related.

The boys constitute a major part of the investigated psychosis group as compared with the remaining material. For the entire material exogenous moments have to a considerably greater extent been indicated as cause than in psychoses, where the illness as such has been a dominating cause of the suicidal attempt. Depressive reactions have dominated the mental state in the attempted suicide both in schizophrenia and in manic-depressive psychosis. Changed behaviour during three months preceding the suicidal attempt has been found to be more frequent in psychoses than in any other diagnosis. Active methods are more often used in the suicidal attempt in schizophrenia than in manic-depressive psychosis and than in the entire material.

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# Renal Response to Intravenous Phosphate Load in Idiopathic Hypoparathyroidism and Pseudohypoparathyroidism

by A. APERIA, O. BROBERGER, C. G. BERGSTRAND and R. ZETTERSTRÖM

It is well established that tubular reabsorption of phosphate is increased in hypoparathyroidism and pseudohypoparathyroidism. Parathyroid hormone is under normal conditions believed to inhibit tubular transport of phosphate. Thus increased reabsorption has been ascribed either to a lack of hormone or an unresponsiveness of the endorgan.

A simple and reliable method of studying the renal reabsorption of phosphate and more specifically determining the renal phosphate threshold i.e. the blood level where the maximal capacity of the tubules to reabsorb phosphate is exceeded and consequently some of the filtered load is excreted, was described by Anderson in 1955 [1]. The method has been used in studies of i.a. parathyroid disorders and pseudohypoparathyroidism [4, 8, 10, 11].

In the present investigation the renal phosphate threshold has been determined in some children and young adults suffering from disorders with a disturbed calcium and phosphate metabolism. The diagnostic value of this procedure will be discussed and compared to the Ellsworth Howard test.

## Material and Methods

The patients studied were divided into 5 groups.

I. *Idiopathic hypoparathyroidism.* This group comprised three children. For clinical data see Table 1.

II. *Pseudohypoparathyroidism.* In this group were included two siblings, brother and sister, 19 and 17 years of age respectively. Both patients, who are mentally retarded, have a history of repeated tetanic seizures, low serum calcium and elevated plasma phosphate values. The diagnosis was based on the presence of typical skeletal abnormalities and a negative Ellsworth Howard test. As could be expected they did not respond to parathyroid hormone but did well on vitamin D. For further clinical data see Table 1. These patients together with patient C. R. of group I have previously been described in detail [2].

III. *Renal phosphate leakage group.* Three children, two of them suffering from familial vitamin D resistant rickets with hypophosphatemia and one from cystinosis were investigated.

IV. *Control group.* This group comprised 5 children of various ages, none of whom showed evidence of a disturbed calcium or phosphate metabolism and without a history of moniliasis or convulsive disorder. Signs of renal tubular insufficiency were present in two of the patients. One of them

TABLE I *Clinical data on patients suffering from idiopathic hypoparathyroidism and pseudohypoparathyroidism*

| Case | Sex | Age at first symptoms | Initial symptoms | Age at appearance of seizures | First diagnosis | Age when correct diagnosis was made | Treatment |
|---|---|---|---|---|---|---|---|
| Cases of idiopathic hypoparathyroidism | | | | | | | |
| B H | m | 2 y 4 m | Breathing difficulties | 1 y 2 m | Epilepsy | 5 y 3 m Malabsorption | Vit $D_2$ |
| S F | f | 3 m | Seizures | 3 m | Tetany | 12 m<br>Y effect of antirachitic treatment | Vit. $D_2$<br>+<br>PTH |
| C. R. | m | 1 y 2 m | Seizures | 1 y 2 m | Epilepsy | 2 y 10 m Cataract | Vit. $D_2$ |
| Cases of pseudohypoparathyroidism | | | | | | | |
| M B | f | 3 y | Seizures | 2 y | Epilepsy | 12 y Cataract Typical skeletal abnormalities | Vit $D_2$ |
| G B | m | 1 y | Subcutaneous calcifications | 10 y | Localized scleroderma | 10 y Cataract Typical skeletal abnormalities | Vit. $D_2$ |

had a renal glucosuria of the hereditary type, the other had a defect in the concentration-dilution capacity which could be localized to the distal tubule In the remaining 3 patients there was no evidence of renal disease

) V *In addition to these 4 groups* a two years old brother (patient G H ) of one of the patients suffering from idiopathic hypoparathyroidism (patient B H. Table 1) was also studied This patient had never had any tetanic seizures and had a normal blood chemistry including calcium and phosphate levels. Since 16 months of age *however he had been suffering from cutaneous* and pulmonary moniliasis. As myotic infection is a frequent finding in idiopathi hypoparathyroidism and sometimes even precedes other clinical [8] or even blood chemical [9] manifestations of the disease by many years t was suspected that he represented a "latent or "partial form of hypoparathyroidism. It was therefore of interest to know if an increased tubular reabsorption of phosphat could be demonstrated at time when the serum calcium and phosphate level were still normal.

All the studies were performed with the patients in a well hydrated stat This was accomplished by giving water in the amount of 1 per cent of the body weight one hour prior to the study followed by a regular 30 min tea water intake in amounts corresponding t the diuresis. On the day of the study the patients were not allowed any food, otherwise there were no dietary restrictions

In all studies the urine was collected by an indwelling bladder catheter by gentle suprapubic pressure and numerous bladder rinsings with air Blood samples were drawn *from a cub tal vein.*

*Ellsworth H ward test.* The test was performed in the conventional way [6] except that the glomerular filtration rate (GFR) was determined simultaneously with the phosphat excretion in order to exclude any possible influence of parathyroid ex tract on glomerular filtration. For this purpose the patient received a *continuous* intravenous infusion of 10 per cent inulin (Laevosan Gesellschaft) at a rat of 0.01 ml/min/kg body weight, preceded by a prime dose of 0.5 ml/min/kg body weight The

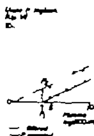


Fig. 1 Implication of the calculation of the theoretical renal phosphate threshold. For explanation see text

infusion was started one hour prior to any determination. Standard learance techniques were used. Following 1 2 control periods, averaging 30 minutes, 200 U of Para-thor mone (Lilly) was given intravenously and the patient was studied for another 3–5 periods of 40–45 minutes.

*Determination of the theoretical renal phosphate threshold.* The procedure followed closely the description given by Anderson in 1955 [1]. Following a 30 minutes control period, when urinary and serum phosphat levels were determined, the plasma phosphat was increased stepwise by the continuous infusion of a 0.27 molar phosphate solution, pH 7.4. The following approxi mat infusion rates were used: 0.025, 0.035 and 0.050 ml/min/kg body weight. Each infusion rate was maintained for t least 20 minutes during which period urinary phosphate excretion was determined. Serial samples for serum phosphate determinations were taken at regular intervals. Sometimes the patients were maintained at the same infusion rat for more than 20 minutes to allow for more urine collection periods. Although the serum calcium tended to fall during the phosphat infusion, there were never any clinical signs of hypocalcemia as judged by the signs of Trousseau or Chvostek or by electrocardiographic recording.

The theoretical renal threshold represents the plasma phosphat level where all the filtered phosphat is reabsorbed. This d termination is obtained by plotting the urinary phosphat excretion, $U V$ against increasing values for plasma phosphat ($Pp$) and extrapolating the line to $UpV = 0$. The great advantage of this method is that the theoretical renal phosphat threshold is independent of glomerular filtration (GFR) which only will influence the slope of the line. An explanation of this is given in Fig 1 where $UpV$ (ordinat ) is plotted against plasma phosphat (abscissa) The broken line represents the theoretical condition that would exist if all the filtered phosphate was excreted i.e $UpV = GFR$ $P_p$. GFR will thus represent the correlation coefficient of the line In reality however a certain amount of filtered phosphate is reabsorbed. Since this amount is rather constant ($= Tm_p$) the true correlation between $U V$ and plasma phosphat will be shifted to the right in a parallell fashion (solid line) According t thi the magnitude of the shift ($=$theoretical renal phosphat threshold) will be an index f the $Tm_p$, the ctual $Tm_p$ being $U V_{pt}$–$C V_{pr}$. The correlation coefficient for the shifted line will naturally also equal GFR.

*Chemical analysis.* Inulin was determined in blood and urin according to the method of Heyrovski [7] Phosphate was determined according t the method of Gomori [6]. The spectrophotometric determinations were carried out with a Beckman-B spectrophotomether

## Results

Fig 2 gives the renal phosphate thresholds in the 5 children of the control group In these children the lowest thres-



Fig 2. The theoretical renal phosphate thresholds in five children of the control group.

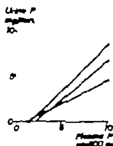


Fig. 3. The theoretical renal phosphat thresholds in three patients of the "renal phosphat leakage group

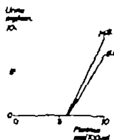


Fig. 5. The theoretical renal phosphate thresholds in two patients with pseudohypoparathyroidism.

hold found was 2.7 mg per 100 ml and the highest 4.2 mg per 100 ml.

The thresholds in the three patients of the "renal phosphate leakage group" are recorded in Fig. 3. As hyperphosphaturia is a dominant symptom both in familial vitamin D resistant rickets and in cystinosis these patients could be expected to have a lowered renal phosphate threshold. All of the three patients had a threshold below that of the controls.

In contrast, an elevated threshold was demonstrated in the patients with idiopathic hypoparathyroidism (Fig. 4) and pseudohypoparathyroidism (Fig. 5). In one of the patients with idiopathic hypoparathyroidism the value was as high as 8.0 mg per 100 ml.

Patient G. H. (younger brother of patient B. H.) who had moniliasis but no other clinical or blood chemical signs of hypoparathyroidism demonstrated a high renal threshold, 5.9 mg per 100 ml (Fig. 6).

Table 2 shows the results of the Ellsworth Howard test in the three patients with idiopathic hypoparathyroidism and the two patients with pseudohypoparathyroidism. It can be seen that all three patients with idiopathic hypoparathyroidism increased their phosphate excretion at least fourfold after intravenous administration of the hormone whereas the two patients with pseudohypoparathyroidism did only double their phosphate excretion. It is of interest that patient G. H. with moniliasis but with normal serum calcium and phosphate levels increased his excretion more than three times.



Fig. 4. The theoretical renal phosphat thresholds in three patients suffering from idiopathic hypoparathyroidism.

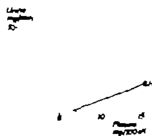


Fig. 6. Theoretical renal phosphate threshold in pt. G.H. a younger brother of pt. B.H. suffering from idiopathic hypoparathyroidism.

TABLE ... *Results of Ellsworth-Howard test in the patients with idiopathic hypoparathyroidism and pseudohypoparathyroidism*

| | GFR ml/min. | P excretion mg/100 ml filtrate |
|---|---|---|
| B. H. 8 y Idiopathic hypoparath. | | |
| Control period 30 min. | 43 | 0.44 |
| 0–30 min. p. PTH* | 61 | 1.07 |
| 30–60 min. p. PTH | 46 | 1.43 |
| 60–90 min. p. PTH | 30 | 1.98 |
| S. F 18 m. Idiopathic hypoparath. | | |
| Control period 30 min. | 10 | 1.4 |
| 0–45 min. p. PTH | 27 | 4.3 |
| 45–90 min. p PTH | 8 | 7.2 |
| C. R. 11 y Idiopathic hypoparath. | | |
| Control period 30 min. | 64 | 0.83 |
| 0–30 min. p. PTH | 35 | 1.7 |
| 30–60 min. p PTH | 45 | 2.5 |
| 60–90 min. p PTH | 43 | 2.0 |
| G H 2 y 6 m. Latent idiopathic hypoparath. | | |
| Control period 30 min. | 19 | 1.4 |
| 0–45 min. p. PTH | 16 | 2.4 |
| 45–90 min. p. PTH | 24 | 4.4 |
| M. B. 20 y Pseudo-hypoparath. | | |
| Control period 30 min. | 64 | 0.67 |
| 0–30 min. p PTH | 33 | 1.38 |
| 30–60 min. p. PTH | 70 | 0.85 |
| 60–90 min. p PTH | 39 | 1.20 |
| O B 17 y Pseudo-hypoparath. | | |
| Control period 30 min. | 114 | 0.25 |
| 0–30 min. p. PTH | 143 | 0.48 |
| 30–60 min. p. PTH | 109 | 1.60 |
| 60–90 min. p PTH | 103 | 0.84 |

* p. PTH After intravenous administration of 200 U of parathyroid extract (Para-thormone Lilly).

The inconvenience in using the total urinary excretion of phosphate as an index of changes in renal phosphate reabsorption in the Ellsworth Howard test is well illustrated in Fig. 7 This figure gives the results of the test in an 18 months

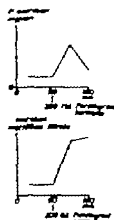


Fig. 7 Ellsworth-Howard test in pt. S F with idiopathic hypoparathyroidism. In the two curves time (abscissa) is plotted against total urinary excretion of phosphate (upper graph) and urinary excretion of phosphate per 100 ml filtrate (lower graph)

old girl with a well established diagnosis of idiopathic hypoparathyroidism. A comparison of the total urinary excretion of phosphate with the excretion per 100 ml filtrate shows a marked discrepancy After administration of parathyroid hormone there was only a temporary increase in the total phosphate excretion, a response that does not differ significantly from what can be obtained in normals or even in patients with pseudohypoparathyroidism However when the phosphate excretion is given as phosphat excretion per 100 ml filtrate a marked and long lasting (up to more than 90 minutes) effect of parathyroid hormone on the reabsorption of phosphat can be noted. Here administration of parathyroid hormone decreased the glomerular filtration rate nd thus totally masked the effect on tubular reabsorption. As seen from Table an ncrease in glomerular filtration rate was observed in some of the other patients.

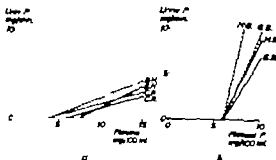


Fig 8. Theoretical renal phosphate threshold before and after intravenous administration of parathyroid hormone in two patients with idiopathic hypoparathyroidism ( ) and two patients with pseudohypoparathyroidism (b) The dotted lines represent the curves obtained after administration of the hormone and the solid lines those obtained before administration of hormone

Fig 8 gives the thresholds before and after administration of parathyroid extract in two patients with idiopathic hypoparathyroidism (a) and in two patients with pseudohypoparathyroidism (b) In this test the extract was administered intravenously 60 minutes prior to the infusion of phosphate It can be seen that the two patients with idiopathic hypoparathyroidism lowered their renal phosphate threshold to a value within the normal range whereas the threshold in the two patients with pseudohypoparathyroidism remained unchanged.

## Discussion

The results clearly demonstrate that determination of the renal phosphate threshold may be a valuable tool in the diagnosis of disorders involving renal tubular handling of phosphate such as idiopathic hypoparathyroidism and pseudohypoparathyroidism.

The method may be of particular value in subclinical cases with normal blood chemistry as illustrated by the findings in patient G H. (cf. Material and Methods) The high threshold 5 0 mg per 100 ml, found in this patient is entirely in agreement with the findings in patients with manifest hypoparathyroidism That changes in renal transport of phosphate are detectable before any abnormalities in the blood levels of calcium or phosphate arise may be related to the fact that inhibition of tubular reabsorption of phosphate is only one of several biological effects mediated by the parathyroid hormone. It may well be that an abnormal parathyroid activity first and more readily manifests itself by a disturbed tubular function. Such an explanation could agree with the findings of Hyde *et al.* [10] in cases of hyperparathyroidism who only have renal calculi but no bone disease or abnormalities of serum calcium or phosphate levels. Thresholds of 2 mg per 100 ml or lower were found in such patients.

As illustrated by the findings in the "renal phosphate leakage group" low thresholds are found in patients suffering from familial vitamin D resistant rickets and other disorders associated with renal tubular dysfunction.

From the data presented, it is obvious that determination of the renal phosphate threshold will more clearly differentiate the various defects in renal phosphate reabsorption than the conventional Ellsworth Howard test, even if due consideration is taken to the glomerular filtration rate This is well demonstrated in the two patients with pseudohypoparathyroidism, where the Ellsworth-Howard test yields a certain increase in phosphate excretion following the administration

of parathyroid extract a finding which might be interpreted as a normal effect On the other hand, the renal phosphate threshold is refractory to the administration of ordinary doses of parathyroid extract. This discrepancy cannot be completely explained by the data available It is, however likely that during the phosphate infusion there is an even rise in plasma phosphate concentration, whereas under the condition of the Ellsworth Howard test spontaneous fluctuations of as much as ±20% are quite common. With such great fluctuations in the plasma phosphate level calculations of the filtered load of phosphate will be rather unreliable The value of the Ellsworth Howard test thus appears to be limited even if consideration is taken to the glomerular filtration rate It therefore seems justified to suggest that calculation of the theoretical renal phosphate threshold may be a useful test in patients where abnormalities in renal phosphate transport are suspected but cannot be proved by other means.


## Summary

The normal range for the renal phosphate threshold in children was found to correspond to that reported for adults In children and young adults with idiopathic hypoparathyroidism or pseudohypoparathyroidism the threshold was found to be abnormally high. Calculation of the renal phosphate threshold seems to be of value for the diagnosis of subclinical cases of idiopathic hypoparathyroidism without tetanic symptoms and with normal blood chemistry

Estimations of the renal phosphate threshold before and after intravenous administration of parathyroid extract was found to be superior to the Ellsworth-Howard test in the diagnosis of disorders associated with renal tubular handling of phosphate such as idiopathic hypoparathyroidism and pseudohypoparathyroidism.


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*Key words*: Renal phosphat threshold, hypoparathyroidism, pseudohypoparathyroidism, Ellsworth Howard test

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# Endogenous Formation of Carbon Monoxide in Newborn Infants

## II. *Rh Haemolytic Disease of the Newborn*

by S. P. FÄLLSTRÖM and J. BJURE

Following Sjöstrand's report 1949 [23] demonstrating increased concentrations of carboxyhaemoglobin (COHb) in adults with haemolytic diseases several reports have appeared on this subject [9, 10, 14, 20]. In haemolytic disease of the newborn, Bakowa & Szczepkowski have found increased concentration of COHb [?]. This finding has later been confirmed by Oski & Altman [19]. In none of these two reports has the relationship between the COHb concentration and the severity of the disease been evaluated in more detail.

The aim of the present study was to test further the hypothesis, that the COHb level reflects the degree of haemolysis in cases of haemolytic disease of the newborn due to Rh isoimmunization. In such cases it is usually not practicable to measure directly the life span of the erythrocytes and one has to rely on indirect criteria of increased erythrocyte destruction, such as hyperbilirubinaemia, anaemia and reticulocytosis. Thus in the present study the COHb level was correlated with the concentrations of bilirubin and haemoglobin and with the number of reticulocytes. Furthermore an attempt was made to define the predictive value of the COHb level with respect to the need for exchange transfusions.

### Material

The COHb concentration was determined in 59 cases of Rh haemolytic disease of the newborn. In 58 cases the maternal serum contained anti D, with in addition, anti-C in nine cases and anti E in two cases. In one case anti E alone was found. All infants were Rh positive with positive direct antiglobulin reaction.

Two infants were delivered at the gestational age of 37 weeks, 10 at 38 weeks, 24 at 39 weeks, 16 at 40 weeks and 6 at 41 weeks. In two cases information on the expected date of delivery was lacking. No infant was premature by weight, but six weighed less than 3000 g. Labour was induced in 40 cases by means of synthetic oxytocin.

Since volatile anaesthetics such as diethylether and trichlorethylene given during delivery have been found to influence the COHb determinations in cord blood and during the first hours of life (unpublished findings), no COHb values from cord blood or the first day of life were included, if these anaesthetics had been used during delivery. On the other hand infants were studied irrespective of the use of nitrous oxide anaesthesia [3].

Most infants received 1 mg Vitamin $K_3$ (menadiolsodiumsulphate). No infant received more than 5 mg.

In two cases there were signs of intrauterine asphyxia (heart rate 80–100 per minute) but none of them had extrauterine asphyxia. One infant had slight extrauterine asphyxia with normal breathing after three minutes. No infants with severe asphyxia and protracted impaired vitality were included in this study since reduced pulmonary function could influence the CO elimination and the COHb level in blood (see below).

Only infants of non-smoking mothers were investigated (see below).

At this paediatric department the following laboratory findings are regarded as signs of haemolysis necessitating early exchange transfusion in Rh haemolytic disease: bilirubin concentration above 3.5 mg/100 ml or haemoglobin concentration below 13.5 g/100 ml in cord blood, bilirubin concentration exceeding 10 mg/100 ml before 1 hours or 15 mg/100 ml before 24 hours of age. These signs of haemolysis were found in 30 infants (group I, filled symbols in figures). The remaining 49 infants (group II, open symbols) did not fulfill the above mentioned criteria. In 16 of these infants the bilirubin concentration later surpassed 20 mg/100 ml.

## Methods

Cord blood was obtained by puncture of the umbilical vein on the placental side after rapid clamping of the umbilical cord. Later venous blood was obtained by puncture of the internal jugular vein or at the beginning of exchange transfusion, through the catheter in the umbilical or saphenous vein.

The carbon monoxide content of the blood was determined according to Linderholm *et al.* [16] after releasing the CO with sulphuric acid in an extraction chamber and analysing the extracted gas in a Hopcalit CO meter (Stålex). The per cent of COHb was calculated from the CO content and the haemoglobin concentration, using 1.34 for the CO combining power of haemoglobin. The random error of the method in the actual range was determined to be ±0.05 per cent COHb.

The methods for determination of haemoglobin and bilirubin concentrations have been described in a previous report [3]. The per cent of reticulocytes was counted on a smear after vital staining for 20 minutes with brilliantcresylblue, at least 1000 erythrocytes being counted. Blood grouping and serological tests were carried out at the blood bank of the hospital. The maternal antibodies were in all cases demonstrated by papain technique and in most cases also by albumin method. The direct antiglobulin test was performed with test serum produced at the blood bank and used routinely in dilution 1:20–1:40. In doubtful cases the reaction was checked with commercial test sera.

Ordinary methods were used for the statistical description of the result [28]. When groups of values were compared, the Wilcoxon two-sample-rank test was applied, since a normal distribution could not be assumed. If no significant difference was found by the Wilcoxon test and the distribution might be the normal one, a t-test was also applied. However in these cases the result of the two tests agreed. A five per cent significance level was used ($p < 0.05$).

## Results

The COHb level in infants with Rh haemolytic disease was compared with that in healthy newborns. Values for COHb in normal newborn infants have been presented previously [3]. The material has subsequently been revised and extended, and consists of 4 healthy fullterm infants without Rh isoimmunization and ABO-incompatibility and without appreciable jaundice (measured bilirubin concentration below 10 mg/100 ml). The number of determinations, mean values and standard deviations at different ages

TABLE 1 *COHb concentration in Rh haemolytic disease and in normal newborns*

| | Rh haemolytic disease | | | | Normal newborns | | |
|---|---|---|---|---|---|---|---|
| Age | group | n | mean | S.D | | mean | S.D |
| Cord blood | I | 10 | 1.83 | 0.83* | 12 | 1 12 | 0 14 |
| Cord blood | II | 5 | 1 13 | 0 08 | 12 | 1 1 | 0 14 |
| 1 12 hours | I | 4 | 2.04 | 0 96 | 13 | 1 03 | 0 15 |
| 13– 4 hours | II | 13 | 1 15 | 0.26 | 8 | 1.02 | 0 15 |
| 25–48 hours | II | 8 | 1 4 | 0.43* | 12 | 0.92 | 0.13 |
| 49– 2 hours | II | 8 | 1 06 | 0 40* | 14 | 0 83 | 0 11 |
| 73–96 hours | II | 11 | 1 46 | 0.89 | 1 | 0 77 | 0 13 |
| 97 144 hours | – | – | – | – | 11 | 0 4 | 0 10 |

* Under the hypothesis that COHb concentrations in Rh haemolytic disease and in normal newborns are equal, the probability is less than 0 05 that differences as large as the observed would be obtained by random.

are shown in Table 1 In the figures mean values are connected by solid lines and 95 per cent population limits (± 2 S.D ) by broken lines. With the exception of cord blood values, each infant is represented by one value only

Cord blood COHb concentrations in 15 infants with Rh haemolytic disease are shown in Fig. 1 In 9 of 10 infants belonging to group I the COHb values exceeded the mean value for normal newborns, in four by more than two standard deviations. The difference between cord blood COHb in group I and in normal newborns was statistically significant (Table 1). In contrast cord blood COHb in five infants belonging to group II did not differ significantly from that in normal newborns (Fig. 1 Table 1) Cord blood COHb concentration in infants with Rh haemolytic disease was significantly correlated to the bilirubin concentration in cord blood ( = 0 73) and to the rate of bilirubin increase during the first 24 hours ( = 0 69) (Table 2) The negative correlation ( = −0.50) between COHb and haemoglobin concentration in cord blood was not significant

Twenty two of 30 infants in group I studied during the first day had COHb concentrations exceeding the mean value for

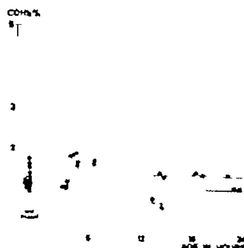



Fig. 1 COHb concentration in cord blood and during the first days of life Rh haemolytic disease Only one value from each infant is represented ... double cord blood values. Filled symbol group I open symbols group II (see text ) Mean values for normal newborns are connected by solid line ... represent ... standard deviations by broken lines.

TABLE 2 *Correlations between COHb and other haematological data in Rh haemolytic disease expressed in correlation coefficients (r)*

| | Regressand | | |
|---|---|---|---|
| Regressor | COHb cord blood | COHb 1–12 hours | rat of bilirubin increase 1st day |
| COHb cord blood | — | | 0.59* (n = 13) |
| COHb 1–12 hours | | — | 0.43 ( = 25) |
| bilirubin cord blood | 0.73 (n = 18) | 0.54 ( = 27) | |
| haemoglobin cord blood | −0.50 ( = 14) | −0.58* (n = 26) | |
| bilirubin 1–12 hours | | 0.62* (n = 24) | |
| capillary haemoglobin | | −0.60* ( = 27) | |
| reticulocytes | | 0.56* (n = 27) | |

The hypothesis that ρ equals zero is rejected at the five per cent level.

normal newborn infants by more than two standard deviations (Fig 1) implying a significant difference between the two groups. (Table 1) Among 16 infants in group II studied during this period, only three had COHb values exceeding the above mentioned limit (Fig 1). The COHb values in group II did not differ significantly from those in normal newborns (Table 1) Infants followed with repeated analyses during the first hours of life are shown in Fig 2 The increasing COHb level in group I was evident, in contrast to the slight decrease found in normal newborns (Fig 1 Table 1) Thus, slight to marked increase was found in all 12 infants in group I. The probability that this would occur by chance is less than 5 per cent (sign test) The COHb concentration in Rh haemolytic disease during the first 12 hours was significantly correlated to the capillary haemoglobin concentration ($r = -0.60$) to the percentual number of reticulocytes ($r = 0.56$), to the bilirubin concentration ($r = 0.62$) and to the rate of bilirubin increase ($r = 0.43$) (Table 2) The COHb concentration during this period was also significantly correlated to the haemoglobin concentration ($r = -0.58$) and the bilirubin concentration ($r = 0.54$) in cord blood.

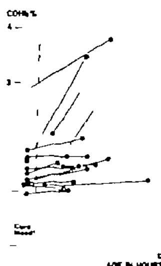



Fig 2. COHb concentration in infants with Rh haemolytic disease followed with repeated determinations during the first 12 hours.

Although fulfilling the criteria for exchange transfusion, five infants in group I were not treated by early exchange transfusion. Their COHb concentrations are shown in Fig 3 The two infants followed by repeated analyses were both later transfused because of haemoglobin concentrations of 7.4 and 9.0 g/100 ml at the age of 5 weeks and 17 days respectively In one of the three remaining infants exchange transfusion



Fig. 3. COHb concentration in five cases in group I (see text), who were not treated by early exchange transfusion.

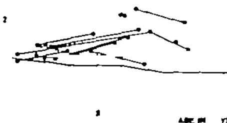


Fig. 4. COHb concentration in infants belonging to group II (see text), with bilirubin concentration exceeding 20 mg per 100 ml.

was performed at the age of 44 hours because of hyperbilirubinaemia.

After the first day the COHb concentration in group II differed significantly from that in normal newborns (Table 1). Fig. 4 shows the COHb values in those 16 infants in group II, who had a bilirubin concentration above 20 mg/100 ml. After the first day of life their COHb concentration, on one or more occasions exceeded the mean level for normal newborns by more than two standard deviations. Five infants followed with repeated analyses from the first day showed a rising COHb level. In Fig. 5 are shown the COHb concentrations in the remaining 13 infants in group II, who did not reach bilirubin concentration of 20 mg/100 ml. Also some of these infants had elevated COHb concentrations after the first day.

During this period significant correlation ($r = 0.65$, $n = 20$) was found between COHb and bilirubin concentrations.

## Discussion

Sjöstrand 1949 reported increased COHb concentrations in adults with haemolytic diseases [23], and could later show that CO is formed during the degradation of haemoglobin [4, 25, 26, 27]. He demonstrated a correlation between the amount of CO eliminated in the expired air and the amount of degraded haemoglobin [27]. The relationship between the alveolar CO concentration and the COHb concentration in blood was also evaluated [5, 22]. Sjöstrand's results were subsequently confirmed by other investigators [6, 7, 9, 17]. The influence of different factors on the COHb level in blood has been discussed in detail by Coburn *et al.* [8], who also stressed the importance of alveolar ventilation and exogenous CO.

In the present study we tried to reduce the influence of exogenous CO by exluding infants of mothers who were smokers since CO is known to pass the placental barrier [11, 13]. The concentrations of CO in air found in this hospital have not been of that order to explain the increased COHb found in haemolytic disease of the newborn. This matter will be discussed further in a subsequent report.

In agreement with the observations of Bakowa & Szczepkowski [2] and Oski & Altman [10] we found markedly increased COHb values in haemolytic disease of

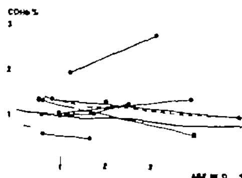


Fig. 5 COHb concentration in infants belonging t group II (see text) with bilirubin concentration below 20 mg per 100 ml

the newborn. Our values were in the same range as those in the first report. Oski & Altman, however as a rule found considerably higher COHb concentrations. They used a spectrophotometric method [4] which in adults gave 1–2 per cent higher COHb concentrations than the infrared CO method. The Hopcalite method [16] used in the present study gave in healthy non-smoking adults COHb values agreeing with those of the infrared method [12]

In infants with Rh haemolytic disease with signs of increased haemolysis immediately after delivery (group I) an increasing COHb concentration was found during the first hours of life (Fig 2) Carbon monoxide passes the placental barrier in both directions [11 13]. An increased elimination of carbon monoxide through the lungs has been found in pregnant Rh isoimmunized women whose infants subsequently were found to have a severe haemolytic disease [15] An efficient CO elimination through the placenta would therefore be a conceivable explanation for the only moderately increased COHb concentrations found in cord blood (Fig 1) After birth CO is eliminated through the lungs of the infant and the efficiency of the infant s pulmonary function will influence the COHb level. The increase of arterial oxygen tension and pH after birth will also influence the COHb level. In normal newborns the effect of all these factors seems to be if any a decreasing COHb level (Fig 1 Table I). Although no infants with signs of respiratory disease were included in the present material, we cannot exclude that minor disturbances in the adaptation to extrauterine life occurred and that a part of the COHb increase after birth might be due to a diminished elimination. But there is no reason to believe that an impaired CO elimination can be the main cause for the rising and/or elevated COHb concentrations found during the first hours after birth. Nor are the COHb values unduly high, but are reasonable, when related to the erythrocyte survival in Rh haemolytic disease [18] and to the COHb in adults with haemolytic diseases [9 10 20] Therefore it can be concluded that the rising COHb concentration after birth in Rh haemolytic disease is a reflection of an increasing rate of erythrocyte destruction. This conclusion is also supported by the significant correlations between COHb and other haematological data

As to the rising and/or elevated COHb concentration after the first day of life in several of the infants with absent or slight signs of increased haemolysis during the first day (group II) it should denote an increasing rate of erythrocyte destruction also in this group In these infants the haemolysis apparently occurred later than in the preceding group. The finding is consistent with the accelerating

rate of bilirubin increase which can be found in Rh haemolytic disease after the first day

According to Poláček [1] there is a good correlation between the bilirubin concentration in cord blood and the subsequent rate of bilirubin increase in Rh haemolytic disease The present investigation also demonstrated a significant correlation between COHb in cord blood and the rate of bilirubin increase Although the limited number of values do not permit a detailed evaluation of the predictive value of a cord blood COHb a concentration exceeding the normal mean value by more than two standard deviations probably is indicative of a severe haemolytic disease On the other hand a COHb concentration not exceeding this limit may be found in infants with marked haemolysis. When cord blood is not available for analysis, an isolated bilirubin value related to age usually gives an approximate estimate of the rat of bilirubin increase The COHb concentration during the first 1_ hours after birth was significantly correlated to the rate of bilirubin increase (Table ) The calculated linear correlation ($r=0\ 43$) however significant was not high but nevertheless the predictive value of the COHb concentration during this period seems to be good. Thus, in 17 of 1 cases with COHb concentrations exceeding the mean value for normal newborns by more than two tandard deviations the bilirubin concentration increased more than 0.5 mg/100 ml per hour which often is regarded the critical rate [1 20]. On the other hand, bilirubin increase was less than 0.5 mg/100 ml per hour in four cases with COHb values below the above mentioned limit In t o other cases not included in the correlation because bilirubin determinations were not available during the first day with low COHb concentration during this period (1–1° hours) bilirubin concentrations determined at 25 and 07 hours of age respectively excluded a bilirubin rise above 0.5 mg/100 ml per hour As to the COHb values during 13–24 hours after birth, data about the bilirubin level are incomplete and do not permit statistical analysis of the association between the COHb concentration and the subsequent bilirubin increase One case in group I had markedly increased COHb associated with a rapid bilirubin rise The three cases in group II with COHb values exceeding the mean value for normal infants by slightly more than two standard deviations reached bilirubin concentrations above "0 mg/100 ml after a few days In only two of eleven infants with COHb values below that limit the bilirubin level subsequently exceeded "0 mg per 100 ml. Thus, a COHb concentration during the first day exceeding the normal mean value by more than two standard deviations, usually means a rapid bilirubin increase A lower COHb concentration seems to exclude a rapid rise of the bilirubin concentration, but not hyperbilirubinaemia during the following days

The COHb level was found to correlate significantly with several haematological parameters used in the evaluation of Rh haemolytic disease Although none of these is a direct measure of erythrocyte survival w believe that there is a relation between the COHb level and the degree of haemolysis in haemolytic disease of th newborn, s in adults with haemolyti diseases [10].

## Summary

The carboxyhaemoglobin (COHb) concentration was determined, once or repeatedly in 50 infants with Rh haemolytic disease of the newborn.

When other laboratory values suggested definitely increased haemolysis COHb values exceeding the mean value for normal newborns by more than two standard deviations were found in four of ten infants in cord blood and in 22 of 26 infants during the first day In this group infants followed with repeated analyses demonstrated an increasing COHb level. In infants with absent or slight signs of increased haemolysis in cord blood and during the first day the COHb concentration exceeded the above-mentioned limit in none of five cases in cord blood and in three of sixteen cases during the first day After the first day however several infants in this group had elevated COHb concentrations

A significant correlation was found between the COHb and bilirubin concentrations in cord blood, and between cord blood COHb and the rate of bilirubin increase during the first day of life. The COHb concentration during the first 12 hours of life was significantly correlated to the bilirubin level, to the rate of bilirubin increase and to the per cent of reticulocytes. A significant negative correlation was found between the COHb and haemoglobin concentrations at this age Furthermore there was a significant correlation between COHb and bilirubin levels after the first day

The results indicate that the relation between the COHb level and the degree of haemolysis found in adults with haemolytic diseases, is also valid during the newborn period.

Although many factors may influence the COHb and bilirubin levels in haemolytic disease of the newborn, the correlation between them is sufficiently good to permit conclusions about the risk of hyperbilirubinaemia from a COHb determination.

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# Studies on Erythro-Kinetics in Infancy

## VI A Method for the Quantitative Estimation of Pulmonary Excretion of Carbon Monoxide in Infancy

by LARS WRANNE

The rate of endogenous formation of carbon monoxide (CO) has been shown to be an index of haem catabolism in the adult individual [6 7 11] In newborn infants too a close connection between CO formation and haemolysis has been claimed [2 9] although the CO formation was studied indirectly by determining the CO content of circulating blood. The following is a description of a method, by which the expired air can be collected and its CO content determined quantitatively in the newborn infant

### Collection of expired air

Expired air was collected in rubber bags during 5-minute periods using the breathing valve described. Samples were mostly obtained from the sleeping infant In some cases, a teat had to be used to prevent mouth breathing

*The breathing valve.* The valve was essentially of the same design as that described by N lson *et al.* [8] but the end tidal sampler of their apparatus was excluded. Basically it consists of two nose-pieces, fashioned to fit into the infant s nares, suitable connections and tubing and two gravity-dependent one-way valves. The latter were made of thin membranes of stainless steel. The breathing valve is shown in Fig 1 The total dead space f the valve, measured with water is about 1.2 ml, varying slightly with different nose-pieces.

The resistance curve for the system is shown in Figure 2. The pressures were estimated with an electromanometer At the usual peak expiratory flow for infants (60 ml/sec) the pressure difference is 9 mm $H_2O$ and t average flow rates (*0 ml/sec) only 3 mm $H_2O$ At these low pressures, back leakage could not be prevented completely However as is shown in Fig. 2, the back leakage at 9 mm $H_2O$ is only 1–1.5 ml/sec and at 3 mm $H_2O$ less than 0.5 ml/sec

The resistance of the valve and tubing was also studied in actual use. Intraoesophageal pressure changes were recorded in one-week-old full term infant during free breathing and during breathing through the valve A water filled polyethylene infant feeding tube was placed in the oesophagus and pressure changes recorded with an lectromanometer During free breathing, the average oesophageal pressure changes were 28 mm $H_2O$ and during valve breathing 32 mm $H_2O$

The present valve's small dead space and low respiratory resistance compare f vour-

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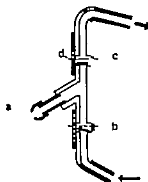



Fig. 1 The breathing valve The valve is made of glass and plastic tubes and stainless steel membranes. - nose piece, b - intake valve - exhaust h d - thin wire which restricts the movement of the membrane

ably with those of valves used by other workers [8, 10, 1 ]. It is essential, in the determination of the expired CO that the respiration is not changed by the breathing valve used. The properties of the present lve seem t guarantee that such changes will not occur to any significant degree

## Estimation of Carbon Monoxid

*Principle* The colorimetric method described by Andersson & Dahlström [1] and by Dahlström [5] was used with some modifications. The unknown gas sample is sucked through a tube containing the indicator gel, which is a mixture of silica molybdenum compound, $H_4Si(Mo_2O_7)_6$ and palladium sulphat $PdSO_4$. Upon contact with carbon monoxide the colour of the indicator gel changes from yellow via green to blue The gel is protected by two layers of moisture-absorbing gel and the tubes re delivered sealed-off The colour change is registered by spectrophotometer The sensitivity of these indicator t bes allows the detection and estimation of carbon monoxide in concentrations less than one part per million (< 1 ppm)

*Apparatus* A Beckman spectrophotometer type B connected to a potentiometric recorder is adapted for CO studies as follows. The standard cell holder is modified to accomodat the indicator t be[1] horizontally in the light-path between the exit slit f the monochromator and the photot be To increase sensitivity the load resistance is increased to *000 megohms. The gas sample is sucked through a water-absorbing gel, the indicator tube and a flow meter by a suction pump. Since the rate of colour-change has been found to be temperature-dependent two additional arrangement are made

[1] Indicator Tubes are manufactured by LKB-Produkter AB, Stockholm 12, type number 3206 A.

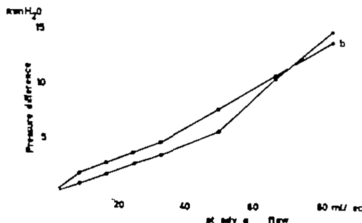



Fig 2 Air resistance of breathing-valv exhaust alv b in ake alve back leakage (same result in both al es)

TABLE 1 *The influence of spectrophotometer cell compartment temperature on CO analyses*

| Temperature C | CO concentration, ppm true | found | |
|---|---|---|---|
| 23 | 1 1 | 0.85 | a |
| 26 | 1 1 | 1.1 | a |
| 29 | 1 1 | 1.3 | a |
| 23 | 6.0 | 5.5 | b |
| 26 | 6.0 | 6.0 | b |

a 8 % increase per 1°C
b 2 % increase per 1°C

Excessive heating of the cell compartment by the tungsten lamp is avoided by air cooling with an electric fan and a water jacket is built into the tube-holder to allow the circulation of constant-temperature water After these precautions, the temperature of the cell compartment can easily be kept at 26 ± 0.5°C. The error thus introduced is about ±4 % (Table 1)

*Procedure.* The indicator tube is connected to the gas sample bag by rubber tubing and *further connected via the flowmeter to the* pump The spectrophotometer is adjusted to zero absorbance the pump started the gas velocity adjusted t 100 ml/minute, and decrease of light transmission t 680 mμ led (Fig 3) Since the first few minutes dings have been found to be unreliable measurements are made between 5 and 15 minutes after starting the gas flow Thus, the minimum volume of the gas sample is 1500 ml.

In order to study the proportion f CO absorbed in the indicator tube the following experiment was carried out. A gas sample with known CO content was sucked through an indicator tube and after that analysed in the usual way by a second indicator tube. The remaining CO concentration was 68 % of the original one. At lower flow rates, the absorbed proportion of CO was higher The fairly high flow rate used 100 ml/minute, was found necessary to ensure an even colour development of the whole length of the indicator gel

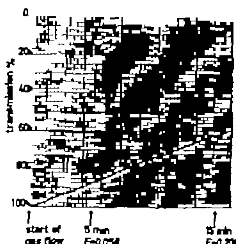



Fig 3. Decrease of transmittance during analysis. Sample contains 5 ppm CO Extinction increase 5–15 minutes = 0.150.

*Calibration* Gas samples of known CO content were obt ined as follows. A standard gas, containing 100 ppm CO in air was measured with a calibrated syringe and mixed with dilution air" which was saturated with water t room temperature The dilution air was obtained at a considerable distance from CO-sources such as combustion engines and heated buildings. Despite this precaution, the dilution ir was found to have apparent CO content of 0.3–0.5 ppm, which had to be allowed for in the calculations of CO content in calibration gas samples. This apparent CO content could be liminated completely by letting the air pass through several columns filled with hopkalit a carbon monoxide combusting catalyst. This was not done in practice, however

In Figure 4, the increase in light extinction between 5 and 15 minutes is plotted against CO concentration. As can be seen, an almost linear relationship is found. One part of CO in one million parts of air corresponded to an increase in light extinction of 0 06. For the following reason, correction for varying barometric pressure was found unnecessary At a gi en concentration of CO the colour development rate was found to be directly proportional t the barometric

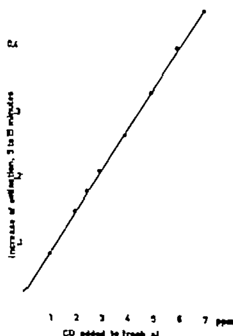



Fig. 4. Increase of extinction of indicator tube between 5 and 15 minutes at different carbon monoxide concentrations. Each point represents the mean of three determinations.

pressure i.e. the colour development was dependent on the absolut amount of CO and not on the concentration. Therefore when the amount of CO expired in unit time is calculated, no correction is necessary

## Estimation of endogenous CO formation

At physiological concentrations of CO in the human body no significant oxidation of CO t CO seems to occur For references see Engstedt [6]. As far as is known, CO can only be eliminated from the body through the lungs and thus, in steady state pulmonary CO excretion equals the endogenous CO formation.

In the present study the pulmonary CO excretion is calculated by the difference between the amount of CO per unit time in inspired and expired ir The lume f inspired air is assumed t be equal t that of expired air In inspired air i.e. room air the CO content was found to vary in the nurseries from 0.2 to 1.3 ppm. There was a conspicuous seasonal trend towards lower values during the summer and higher values during the winter CO concentration could change by up to 0.8 ppm within a few days. Changes within one day were less, not more than 0.2–0.3 ppm. Smoking was not allowed in the nurseries or in their surroundings.

## Error of sampling and CO analysis

*Estimation of expired ai volume.* Double determinations of the minute volume of expired air were performed on 20 occasions in six infants 0–5 days old. The mean minut volume was 535 ml/minut BTPS and the range was 410–690 ml. There was no con sistent difference between the two samples of these double determinations. The standard error f a single determination was ±29 ml or 5.5% of the mean value

The mean value and the range of 90 single determinations were compared to the result f Cook *et al* [3] and of Cross *et al.* [4]. When infants of equal birth weights were compared, the results of the present investigation were found to agree with the earlier studies (Table 2).

*Analysis f CO in room air* Samples of room air from nurseries were btained on twenty different occasions before and after the sampling of expired air from infants. The concentrations ranged from 0.5 t 1.3 ppm and the mean value was 0.8 ppm. The standard deviation f a single determination was ±0 06 ppm. There was no significant difference between the means of the first and second determinations.

*Analysis of CO expired ir* This was performed on the bove mentioned samples. For practical reasons, the second sample could not be obtained immediately after the first The time interval between the two samples did not exceed two hours. The analyses were performed within two further hours. When the means of the first and second nalyses were calculated, no significant differences ppeared. The mean CO concentration f the samples was 2.3 ppm and the

TABLE 2 *Respiratory minute volume in the newborn period Present results compared to those of Cook et al [3] and Cross et al [4]*

| | Number of determinations | Weight of infants kg | Respiratory minut volume ml/min BTPS mean | range |
|---|---|---|---|---|
| Cook et al. | 35 | 1.8–4 1 | — | 353–845 |
| Cook et al. | 7 | 2.1–4.0 | 596 | 481–840 |
| Cross et al. | 47 | 2.4–4.5 | — | 329–684 |
| Cross et al. | 30 | 2.1–4.0 | 510 | 375–684 |
| Present dat | 60 | 2.1–4.0 | 565 | 410–775 |

Infants weighing 2.1–4.0 kg selected in order t correspond to present data.

range 1.5–5.8 ppm. The standard error f a single determination was found to be ±0 1 ppm or 5% of the mean.

*Estimation of CO expired per unit time* was performed on the bove-mentioned samples. The mean was 1.2 microlitres per minut STPD and the range 0 7–3.2 microlitres. The means of the first and second estimations did not differ significantly The standard error of a single determination was ±0.27 microlitres per minut or ±22% of the mean.

## Discussion

In infants, and especially in neonates, it is very important to be able to perform quantitative studies of haemolysis. The common haematological methods, how ever are difficult to use and interpret in this age-group Measurements of the endogenous formation of carbon monoxide first described by Sjöstrand [11] seem to offer an opportunity to study the haemolysis even in the newborn. The present investigation has tried to evaluate the possibilities of studying the endogenous formation of carbon monoxide by measurements of the pulmonary excretion of CO

*Technical problems* The concentration of carbon monoxide (CO) in inspired and expired air is of the order of one or two parts per million. The method now applied determines such concentrations with reasonable accuracy the standard error of a single determination being ±5%.

In order to determine pulmonary excretion of CO per unit time it is necessary to perform a quantitative collection of expired air The technical aids used in this collection must not cause hyperventilation by irritating the infant or by increasing the respiratory dead space Also, leaks must be avoided so that the volume is not underestimated. The procedure should be simple enough to permit repeated investigations. By the present method, collections can be made in the sleeping infant, the dead space is small, and the procedure can be repeated almost indefinitely The random error in minute volume determination is ±5.5%. In comparison with earlier studies of respiratory minute volumes in the newborn (Table 2) losses due to leakage which would, of course give too low values seem no greater in this study and, probably no important losses have occurred.

Thus, the technical problems inherent in determinations of pulmonary excretion of CO are not insurmountable The collec

tion of expired air is time-consuming and the complete analysis of pulmonary excretion of CO is not too precise. However the analysis can be repeated several times.

*Theoretical considerations.* At physiological concentrations, CO is not metabolized in the body and thus CO excretion should closely parallel the endogenous formation. This is not the case however if the body CO pool is changing. The distribution of CO in different organs is not known in detail but it can be safely assumed that the most important pool is that of circulating carboxyhaemoglobin. The size of this pool can be calculated with the aid of the data of Bjure & Fällström [2] and by the blood volume determinations of Usher *et al.* [13]. Accordingly the total CO in circulating blood of a three day-old infant weighing 3.5 kg, amounts to approximately 0 ml. Studies now under way [14] indicate that the corresponding pulmonary excretion of CO is around 40 microlitres per hour. Thus the COHb-pool equals some 18 hours pulmonary excretion.

From the above discussion, it seems clear that determinations of the pulmonary excretion of carbon monoxide can give good estimates of the endogenous formation only if the COHb concentration is stable. However if rapid changes of the endogenous formation and, therefore also of the COHb concentration occur a time lag must be expected before the pulmonary excretion equals the endogenous formation. Also if the pulmonary ventilation or perfusion is changing the possibility must be considered that CO is retained or washed-out from the body COHb-pool. In this case the endogenous formation of CO cannot be estimated without repeated investigations of the COHb concentration as well as of the pulmonary excretion.

In conclusion, studies of the pulmonary excretion of CO seem to be a possible method to be used in a steady state for the quantitative estimation of the endogenous formation of CO and, probably also of the haemoglobin breakdown. However in cases with rapidly changing haemolysis or with abnormalities of pulmonary function, it is necessary also to repeatedly investigate the COHb concentration.

## Summary

A technique is described, by which the pulmonary excretion of carbon monoxide in the newborn infant can be quantitatively determined. The expired air is collected by aid of a breathing valve with small dead space and low respiratory resistance. Th analysis f carbon monoxide is precise en in concentration about 1 part per million.

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# Studies on Erythro-Kinetics in Infancy

## VII Quantitative Estimation of the Haemoglobin Catabolism by Carbon Monoxide Technique in Young Infants

by LARS WRANNE

Hyperbilirubinaemia is a common finding in otherwise apparently normal newborn infants. One possible major cause of this condition is the relative inability of the neonate to conjugate and hence excrete bilirubin. However variations in haemoglobin catabolism may also be important. It is difficult to estimate haemoglobin catabolism by the conventional methods. Studies of the life span of erythrocytes from neonates by the current non-cohort techniques do not give adequate information about the haemolysis during the first week of life

The endogenous formation of carbon monoxide (CO) seems to reflect the haemoglobin catabolism in man [2, 3, 11]. Theoretically 4 molecules of CO are released by the breakdown of 1 molecule of haemoglobin, i.e. 1.34 ml CO per gram haemoglobin. In the present study an attempt was made to estimate the CO excretion in infants 0–5 months of age

It cannot be presumed that the endogenous formation of CO always equals the pulmonary CO excretion. Thus, in the following, the word "excretion" refers to the CO measured, whereas "endogenous formation" refers to estimates of the actual CO production within the body

### Methods

The pulmonary excretion of CO was measured as described in a previous paper [12]. Blood haemoglobin and bilirubin concentrations were determined by the cyano-methaemoglobin method and by Michaëlsson's method [7], respectively. Reticulocyt counts were performed after 20 minutes incubation of erythrocytes in 1% brilliant-cresyl blue in saline

### Material

Healthy neonates were studied from their first or second day of life. Some data concerning these infants are given in Table 1. Coombs direct test on the erythrocytes was always negative. Physical examination showed nothing abnormal.

As a rule CO studies were performed once or twice during the first two days of life and subsequently every or every alternate day up to the age of one week. After this period the mothers and infants were dis-

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TABLE 1 *Clinical and laboratory data of infants studied*

| Name | Birth weight grams | Duration of pregnancy days | Blood group Mother | Blood group Child | Highest bilirubin mg/100 ml[a] | Highest reticulocyte count %[b] | Haemoglobin, day 0–1 g/100 ml[c] |
|---|---|---|---|---|---|---|---|
| *"ABO-compatible"* | | | | | | | |
| Be | 3670 | 290 | A Rh+ | A Rh+ | 6.7 | 0.0 | 22.4 |
| Bj | 3270 | 280 | O Rh+ | O Rh+ | 6.2 | 1.7 | 22.2 |
| Ek | 3150 | 271 | A Rh+ | A Rh+ | 4.2 | 3.1 | 22.2 |
| Er | 3110 | 285 | A Rh+ | A Rh+ | 8.6 | 2.0 | 24.6 |
| Ge | 3260 | 276 | A Rh+ | A Rh+ | 8.2 | 4.0 | 20.1 |
| Is | 3220 | 277 | O Rh− | O Rh− | 9.2 | 2.0 | 22 |
| Jo | 3400 | 300 | A Rh+ | A Rh+ | 6.6 | 1.5 | 24.2 |
| La 1 | 3190 | 278 | A Rh+ | A Rh+ | 7.8 | 5.0 | 23.3 |
| Li | 3790 | 284 | AB Rh+ | AB Rh+ | 2.9 | 2.4 | 18.9 |
| Ob | 3030 | 277 | A Rh+ | A Rh+ | 4.1 | 0.8 | 16.2 |
| Pa | 3090 | 274 | O Rh− | O *Rh*− | *8.5* | *1.5* | *18.9* |
| Pet | 3640 | 280 | AB Rh+ | AB Rh− | 4.6 | 4.0 | 20.6 |
| So | 3350 | 273 | O Rh+ | O Rh+ | 2.2 | 1.2 | 22.0 |
| Sv | 3670 | 278 | A Rh+ | A Rh+ | 6.0 | 4.1 | 24.6 |
| La 2[d] | 2810 | 278 | A Rh+ | O Rh+ | — | — | 22.2 |
| *"ABO-incompatible"* | | | | | | | |
| An | 3700 | 268 | O Rh+ | A Rh+ | 4.8 | 2.0 | 18.4 |
| Bl | 3790 | ? | O Rh− | B Rh+ | 4.5 | 3.1 | 19.9 |
| Er | 3600 | 285 | O Rh+ | A Rh+ | 2.4 | 1.0 | 24.7 |
| J | 3660 | 268 | O Rh+ | A Rh | 3.4 | 4.0 | 21.4 |
| Ka | 3830 | 279 | O Rh+ | A Rh+ | 8.3 | 3.1 | 20.6 |
| La 3 | 4060 | 290 | O Rh+ | A Rh+ | 5.2 | 1.4 | 20.7 |
| Lu | 3120 | ? | O Rh+ | A Rh+ | 3.0 | 4.0 | 21.4 |
| Per | 3250 | 284 | O Rh+ | A Rh+ | 3.0 | 4.0 | 22.2 |
| Po | 2920 | ? | O Rh+ | A Rh+ | 6.3 | 3.3 | 20.1 |
| Ra | 4300 | 288 | O Rh+ | A Rh+ | 3.2 | 2.7 | 21.0 |

[a] Highest bilirubin observed during first week.
[b] Reticulocyte counts on first two days of life not included.
[c] Capillary blood.
[d] Study started 2nd week.

charged from the obstetric department. In some infants, however, further studies could be made at home. The estimation of endogenous CO formation requires a stable environmental CO concentration. The parents were therefore asked to refrain from smoking, or from ventilating the rooms excessively during the hours preceding the study.

When the infants reached the age of 3–5 months, increasing technical difficulties made further studies impossible.

Haemoglobin and bilirubin concentrations in capillary blood from unwarmed heels were determined together with the reticulocyte count at the ages of 0–1 day, 2–4 days and 5–7 days (Table 1). Subsequently only the haemoglobin was measured at each CO investigation (Table 2).

## Results

### First week of life

*ABO-compatible versus ABO-incompatible infants.* In fourteen of the infants the ABO blood groups were compatible

TABLE 2. *Pulmonary excretion of carbon monoxide*

Microliters CO/kg body weight hour

| | Number of examinations | Age (hours) 0–12 | 13–24 | 25–36 | 37–48 | Day of life 3 | 4 | 5 | 6 | 7 | 8 | Mean 0–8 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ABO-compatible* | | | | | | | | | | | | |
| Be | 7 | 17.0 | 15.6 | 4.9 | 7.6 | 10.1 | 12.5 | 6.5 | — | — | — | 10.6 |
| Bj | 7 | 10.9 | 8.0 | 8.9 | — | 4.1 | — | 14.1 | 15.5 | 11.4 | — | 10.3 |
| Ek | 6 | 11.8 | — | 9.6 | — | 12.0 | 11.3 | 12.9 | — | — | 10.2 | 11.5 |
| Er | 6 | 13.1 | 14.4 | 14.0 | — | 11.1 | 11.4 | 11.3 | — | — | — | 12.5 |
| Ge | 9 | 13.4 | 13.8 | 15.1 | 14.3 | — | 10.5 | 9.6 | 16.5 | 11.0 | 8.8 | 12.2 |
| Is | 6 | 12.3 | — | 10.0 | — | — | 6.8 | 10.1 | 9.5 | 1… | — | 10.2 |
| J | 4 | 14.5 | — | 11.1 | 12.1 | — | — | 8.3 | — | — | — | 11.5 |
| La 1 | 8 | — | 16.3 | 16.1 | — | 12.3 | 20.1 | 7.0 | 3.5 | 8.0 | 5.8 | 11.8 |
| Li | 6 | — | 16.6 | 3.6 | — | 5.2 | 6.0 | 4.4 | 10.0 | — | — | 7.7 |
| Ob | 5 | 17.8 | 14.4 | 12.2 | — | 15.9 | — | 7.5 | — | — | — | 13.6 |
| Pa | 6 | 11.7 | 8.9 | 11.2 | — | 11.6 | 13.1 | 10.4 | — | — | — | 11… |
| Pet | 5 | 13.8 | — | 14.3 | — | — | — | 7.3 | 9.1 | 16.6 | — | 12.2 |
| Se | 6 | 13.4 | 9.4 | 10… | — | 9.0 | — | 5.7 | 6.1 | — | — | 9.0 |
| Sv | 7 | — | — | 18.0 | 15.6 | 8.0 | 16.1 | 14.9 | 11.4 | 17.5 | — | 14.1 |
| Number of examinations 88 | | 11 | 9 | 14 | 4 | 10 | 9 | 14 | 8 | 9 | | — |
| Mean | | 13.5 | 13.0 | 11.3 | 12.4 | 10.0 | 12.1 | 9.3 | 10.2 | 11.3 | | 11.3 |
| *ABO incompatible* | | | | | | | | | | | | |
| An | 6 | — | 9.0 | 12.6 | 10.4 | 9.5 | 13.4 | 10.5 | — | — | — | 10.9 |
| Bl | 9 | 7.7 | 7.3 | 2.7 | 9.4 | 3.0 | 8.7 | — | 9.5 | 9.7 | 3.5 | 7.2 |
| Er | 6 | 21.8 | 11.8 | 12.3 | — | 14.2 | 9.7 | 8.6 | — | — | — | 12.6 |
| J | 7 | 15.0 | 15.8 | 9.3 | 9.1 | 6.4 | 8.4 | — | 8.1 | — | — | 10.4 |
| Ka | 5 | 7.7 | 10.6 | — | 13.2 | 14.4 | 11.6 | — | — | — | — | 11.5 |
| La 2 | 6 | 8.4 | 12.8 | — | 4.0 | 7.7 | 13.6 | 8.9 | — | — | — | 8.7 |
| Le | 6 | 15.7 | 18.9 | — | 17.7 | 11.5 | 10.5 | 8.6 | — | — | — | 13.2 |
| Per | 6 | — | 16.5 | — | 9.9 | 10.5 | — | 5.8 | 17.5 | 17.7 | — | 12.9 |
| Po | 7 | 7.3 | 17.0 | 9.3 | 6.1 | 10.2 | — | — | 7.6 | 4.7 | — | 9.4 |
| Ra | 5 | 11.3 | — | 13.4 | 13.2 | 13.6 | 8.1 | — | — | — | — | 12.0 |
| Number of examinations 63 | | 8 | 9 | 6 | 9 | 10 | 8 | 5 | 4 | 4 | | — |
| Mean | — | 11.9 | 13.0 | 10.0 | 10.1 | 10.1 | 10.5 | 7.2 | 10.7 | 8.9 | | 10.9 |
| Total number of examinations | | 37 | | 33 | | 20 | 17 | 19 | 12 | 13 | | |
| Mean of all determinations | | 12.9 | | 10.9 | | 10.1 | 11.4 | 8.8 | 10.4 | 10.6 | | |

with those of their respective mothers. ABO incompatibility was found in eleven infants. It may be noted that there were no cases of hyperbilirubinaemia in either group. The CO excretion of each infant during the successive days is shown in Table 2 The "ABO-compatible" and ABO incompatible groups of infants showed the same CO excretion, and the two groups were therefore combined.

*CO excretion during the first week.* There appeared to be a slight decrease in CO

TABLE 3 *Pulmonary excretion of carbon monoxide (*) body weight and blood haemoglobin concentration from 1st to 22nd week of life*

| Week of life | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th | 11th |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Be | Weight | 3070 | 2500 | 3700 | 3800 | | | | | 4600 | | |
| | Hb g/100 ml | 23.4 | 19.6 | 19.3 | 17.7 | | | | | 11.4 | | |
| | CO/kg | 10.6 | 7.2 | 7.7 | 7.3 | | | | | 2.9 | | |
| Ek | Weight | 3150 | 3000 | 3000 | 3250 | 3430 | | 3900 | | 4600 | | 5150 |
| | Hb g/100 ml | 22.2 | 22.8 | 18.9 | 15.6 | 16.0 | | 13.6 | | 11.6 | | 11.4 |
| | CO/kg | 11.8 | 2.9 | 4.7 | 10.5 | 7.8 | | 8.4 | | 6.0 | | 4.0 |
| Ge | Weight | 3200 | 3070 | 3180 | | 3400 | 3600 | | 4000 | | 4600 | |
| | Hb g/100 ml | 20.1 | 17.8 | 17.0 | | 16.7 | 15.2 | | 14.2 | | 11.2 | |
| | CO/kg | 12.2 | 3.3 | 4.4 | | 5.4 | 6.0 | | −1.0 | | 5.4 | |
| Is | Weight | 2220 | 3150 | 3250 | 3550 | 3750 | | 4000 | | | 4700 | |
| | Hb g/100 ml | 22.2 | 16.8 | 17.0 | 15.4 | 15.0 | | 16.5 | | | 12.8 | |
| | CO/kg | 10.2 | 6.6 | 2.8 | 11.0 | 10.4 | | 7.4 | | | −_0 | |
| La 1 | Weight | 3190 | | 3200 | 3450 | | 3750 | 4130 | 4410 | | | 4800 |
| | Hb g/100 ml | 26.8 | | 20.0 | 19.2 | | 17.7 | 15.2 | 13.6 | | | 11.6 |
| | CO/kg | 11.8 | | 9.3 | 5.7 | | 6.7 | 12.6 | 3.5 | | | 4.5 |
| La 2 | Weight | 2610 | 2700 | | 2810 | | | | | | | |
| | Hb g/100 ml | 23.3 | 18.0 | | 18.0 | | | | | | | |
| | CO/kg | — | 12.6 | | 7.4 | | | | | | | |
| Li | Weight | 3790 | | | 400 | | 4300 | | | | | |
| | Hb g/100 ml | 19.9 | | | 16.6 | | 16.5 | | | | | |
| | CO/kg | 7.7 | | | 4.2 | | 6.8 | | | | | |
| Ob | Weight | 3030 | 2980 | 3200 | 3400 | | | | | | 4300 | |
| | Hb g/100 ml | 16.2 | 16.8 | 15.4 | 12.5 | | | | | | 14.2 | |
| | CO/kg | 13.6 | 11.3 | 6.7 | 3.8 | | | | | | 1.4 | |
| Pa | Weight | 3090 | | | 3210 | | 3500 | | | | | |
| | Hb g/100 ml | 18.9 | | | 16.8 | | 17.1 | | | | | |
| | CO/kg | 11.2 | | | 1.7 | | 6.6 | | | | | |
| Per | Weight | 3.50 | | 3300 | 3500 | 3700 | | 4000 | | 4600 | | |
| | Hb g/100 ml | 22.2 | | 19.0 | 18.8 | 16.0 | | 12.6 | | 12.8 | | |
| | CO/kg | 12.9 | | 14.1 | 14.4 | −1.7 | | 6.5 | | 1.0 | | |
| Pet | Weight | 3640 | 3500 | 3000 | 3800 | | | 5000 | 5250 | | | |
| | Hb g/100 ml | 20.6 | 19.3 | 16.8 | 16.0 | | | 15.2 | 15.0 | | | |
| | CO/kg | 12.2 | 8.0 | 8.5 | 0 | | | 8.5 | 5.4 | | | |
| Po | Weight | 2920 | | 2990 | 3000 | 3010 | 3200 | | 3450 | | | |
| | Hb g/100 ml | 20.1 | | 19.0 | 13.6 | 12.8 | 12.0 | | 11.3 | | | |
| | CO/kg | 9.4 | | 0.8 | 4.9 | −1.9 | 22.1 | | 7.3 | | | |
| Sa | Weight | 3250 | | 3010 | 3780 | 4000 | 4125 | | | | | |
| | Hb g/100 ml | 22.0 | | 18.6 | 16.6 | 15.0 | 14.9 | | | | | |
| | CO/kg | 9.0 | | 4.2 | 5.6 | 11.9 | 6.9 | | | | | |
| Sv | Weight | 3470 | 3500 | 3920 | 3380 | 4100 | | 4600 | | 5200 | | 5500 |
| | Hb g/100 ml | 24.8 | 20.4 | 17.7 | 15.6 | 17.7 | | 13.4 | | 13.4 | | 11.3 |
| | CO/kg | 14.1 | 12.2 | 9.3 | 11.3 | 6.5 | | 8. | | 2. | | |

| Week of life | | 12th | 13th | 14th | 15th | 16th | 17th | 18th | 19th | 20th | 21st | 22nd |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Be | Weight | | | 5960 | | 6250 | | 6500 | | | | |
| | Hb g/100 ml | | | 12.0 | | 12.6 | | 12.4 | | | | |
| | CO/kg | | | 4.2 | | 4.0 | | −2.2 | | | | |
| Ek | Weight | | | 5000 | | 5800 | | | 6150 | | | |
| | Hb g/100 ml | | | 11.7 | | 12.3 | | | 11.6 | | | |
| | CO/kg | | | 0.0 | | 2.0 | | | 0 | | | |
| Ge | Weight | 5100 | | 5080 | | | | 5800 | | 6470 | | 6800 |
| | Hb g/100 ml | 11.8 | | 12.8 | | | | 14.5 | | 11.9 | | 15.0 |
| | CO/kg | 11.6 | | 0 | | | | 7.5 | | 3.1 | | 2.7 |
| Is | Weight | | 5200 | | | 5700 | | 6100 | | | 6600 | |
| | Hb g/100 ml | | 15.0 | | | 13.1 | | 13.1 | | | 13.9 | |
| | CO/kg | | 1.7 | | | 1.1 | | −8.1 | | | 2.2 | |
| La 1 | Weight | | | | 5700 | | | | | | | |
| | Hb g/100 ml | | | | 13.6 | | | | | | | |
| | CO/kg | | | | 2.5 | | | | | | | |
| La 2 | Weight | 4010 | | | | 4570 | | | | | | |
| | Hb g/100 ml | 13.2 | | | | 13.4 | | | | | | |
| | CO/kg | 4.9 | | | | 2.6 | | | | | | |
| Sv | Weight | | 5900 | | | 6200 | | | | | | |
| | Hb g/100 ml | | 14.0 | | | 18.3 | | | | | | |
| | CO/kg | | 0.9 | | | −.2 | | | | | | |

excretion during the first seven days of life (Table 2) the mean excretion being 11 1 µl CO per kg body weight and hour (11.1 µl/kg × hr) During the first day of life, however the excretion was slightly but significantly higher (13.9 µl/kg × hr) than during the rest of the first week (mean 10 4 µl/kg × hr $p < 0.001$) The CO excretion showed no correlation with the haemoglobin concentration, maximum bilirubin concentration or reticulocyte count.

### *Age 1 week–5 months*

The individual results of CO studies on infants older than one week are found in Table 3 together with data on their body weights and blood haemoglobin concentrations. As could be expected, no fixed time-schedule could be applied to these studies Upper respiratory infections that otherwise were insignificant made the collection of expired air very difficult. In some cases, studies had to be abandoned due to the increase of motor activity of the infants.

In all, fourteen infants were studied on a total of 85 occasions after the first week of life The shortest follow up period was five weeks, and the longest twenty-one weeks. Each infant was studied three to fifteen times.

The mean values of CO excretion and some other data are found in Table 4. As can be seen, the mean CO xcretion decreased from 11.2 µl/kg × hr during the *first* week to 6–9 µl/kg × hr during the following six weeks, and even lower values were obtained after this period, during the 16th to 18th weeks of life the CO excretion averaged only 0.35 µl/kg × hr

The individual result of the CO studies show considerable variations. In some determinations, less CO was exhaled than inhaled. These results are shown in Table 3 as negatives" and are included as such in the calculation of means. The greatest "negative" difference between exhaled and inhaled CO was −0 6 parts of CO per million parts of air

### Discussion

In this study the endogenous CO formation was estimated by analysis of the difference of CO concentration in inhaled and exhaled air and by measurement of the respiratory minute volume. These determinations have a considerable combined analytical error S.D being ±23 % [12]. It may be anticipated therefore that only the calculation of means of a large number of CO values will give significant results. The random variation of the analytical procedure probably also gives the paradoxical "negative" results in some infants 1–4 months of age In these more CO was found in the inhaled than in the exhaled air Since this was felt to be due to such random analytical errors or possibly to random transient variations in the environmental CO concentration, "negative results were included in the calculation of means.

### *Age versus excretion of CO*

A higher CO excretion was noted during the first day of life than during th following days. This finding could indicate that the haemoglobin catabolism is higher during the first day Sjölin [10], on studying the osmotic fragility of red cells in vitro f und population of very fragile cells in cord blood that ppeared to be absent

Table 4 *Carbon monoxide excretion during 1st to 22nd week*

| Week of life | Number of infants | Number of examinations | CO $\mu$l/kg × hr | CO $\mu$l/hr per infant | Calculated haemolysis: mg Hb/24 hrs per kg body weight | g Hb/24 hrs per infant | from birth to end of period g Hb |
|---|---|---|---|---|---|---|---|
| 1st | 24 | 181 | 11.2 | 33 | 185 | 0.62 | 4.3 |
| 2nd | 8 | 8 | 8.3 | 26 | 140 | 0.43 | 7.3 |
| 3rd | 11 | 11 | 6.6 | 22 | 110 | 0.34 | 9.8 |
| 4th | 13 | 13 | 7.1 | 30 | 10* | 0.41 | 12.7 |
| 5th | 7 | 7 | 6.0 | 23 | 100 | 0.35 | 15.2 |
| 6th–7th | 11 | L | 9.0 | 34 | 150 | 0.59 | 23.4 |
| 8th–9th | 8 | 8 | 4.8 | 22 | 80 | 0.36 | 28.4 |
| 10th–12th | 7 | 8 | 5.5 | *0 | 90 | 0.43 | 37.4 |
| 13th–15th | 6 | 6 | 1.5 | 8 | 25 | 0.14 | 40.2 |
| 16th–18th | 6 | 8 | 0.35 | 2 | 6 | 0.03 | 40.8 |
| 19th–22nd | 3 | 4 | 2.0 | 13 | 30 | 0.21 | 46.5 |

in infants aged 1°–48 hours. Destruction of such a population of cells might have contributed to the higher CO excretion. However this excretion may have been increased by a simple change of steady state on the adjustment of the newborn nfants to extrauterine life. In the studies f Bjure & Fallström [1] the mean CO oncentration fell from 1 14 % in cord lood to about 0 9 % in blood taken during he first few days of life Assuming that his excess CO is eliminated during the irst 24 hours, and, further that the ndogenous formation of CO is unchanged, his would imply a mean CO excretion of bout 1.2 $\mu$l/kg × hr In the present study he observed difference between the first ay (12.9 $\mu$l/kg × hr) and the subsequent days (10 4 $\mu$l/kg × hr) was 2.5 $\mu$l/kg × hr This difference thus indicates that an increased destruction of red cells may occur during the first 24 hours of life However the destruction is probably not more than 15 % higher than that found later in the first week.

During the rest of the first week of life the CO excretion changes only slowly which must mean that in the normal infant the endogenous CO formation is fairly stable

From the end of the first week up to the fifth week, the CO excretion appears fairly stable although lower than during the first seven days (Table 4) The moderate increase in CO excretion noted in the sixth and seventh weeks is difficult to interpret It cannot be excluded that this was due only to chance ($p < 0.05$ that CO excretion during weeks 6 + 7 was the same as that during weeks 5 + 8 + 9)

The most striking observation during these studies was the very low CO excretion (0.35 $\mu$l/kg × hr) during the 16th–18th weeks (Table 4) The excretion during the first week was in fact 30 times higher than that of infants in their 16th 18th weeks of life When calculated per kg body weight, the difference was still greater the excretion during the first week now being about 30 times higher

This finding can hardly be explained by any systematic error giving the CO excretion as below the true value but must mean that this excretion and hence the endogenous CO formation is very low at this later age. This does not necessarily indicate that the haemoglobin catabolism is diminished to the same degree. Loumanmäki [6] has studied the endogenous disappearance of $^{14}CO$ from the blood at equilibrium. Although his experiments were performed in dogs and the occurrence of slowly equilibrating CO pools could not be completely excluded, it may be justifiable to calculate the corresponding effect in infants of this age. Loumanmäki found that about 1% of body CO stores disappeared per hour by some extrapulmonary mechanism, part of which was proved to be oxidation to $CO_2$. Assuming the same elimination rate in the infants studied here and assuming that their CO-haemoglobin concentration was about 0.5%, the endogenous disappearance would be about 0.85 µl/kg × hr. The true endogenous formation of CO from haemoglobin catabolism would thus be 1.2 µl/kg × hr but hardly higher. This figure is still 10 times less than that found during the infant first week.

*Probable explanation for low CO endogenous formation during 16th 18th weeks*

Haemoglobin synthesis is very brisk in the newborn infant but decreases rapidly during the first days of life. During the subsequent six weeks, erythropoiesis remains relatively dormant [4], but this is followed by a new period of activity and for some time there are two distinctly different red cell populations: one produced during the foetal period and the first days of extrauterine life and another produced after the age of six weeks. Since it is probable [5] that erythrocyte survival in the neonate is shorter than that of cells with "adult behaviour" an interval of at least six weeks can be expected between the ends of the life spans of the majority of the neonatally and postneonatally formed erythrocytes. A decrease of the endogenous formation of CO could therefore be expected not later than the 17th week, followed by an increase after the 23rd week. It is unfortunate that for technical reasons the present study could not be extended beyond the 22nd week, so that this theory could be proved. However, the rapid decrease in CO excretion noted after the 13th week seems to fit the theoretical considerations. Also this finding suggests that the life span of the majority of red cells of the newborn is less than 13 weeks or 90 days.

*Other CO sources than haemoglobin catabolism at end of red cell life span.* Coburn et al. [*], in adult studies, found that the endogenous formation of CO was 0.4 ml per hour. If calculated from the total blood volume and an erythrocyte life span of 120 days the expected CO formation should only be about 0.20 ml/hour. Factors that can be expected to augment the endogenous formation of CO are early haemolysis of young red cells in the bone marrow or in the peripheral blood, random destruction of red cells, or CO formation from other sources than haemoglobin catabolism. The very low CO excretion found in infants in their 13th to 18th weeks seems to indicate that at this age these factors are fairly unimportant. It may also be concluded that only a minor number of erythrocytes of the second population are hemolysed before the 19th week. Thu

the life span of this population probably exceeds 13 weeks. More detailed information would be obtained by further studies of infants 18–25 weeks of age

*Quantitative relationship between observed and expected CO excretion.*

It has been stressed above that the low CO excretion during the 13th–18th weeks indicates that all cells of the first population of red cells have been destroyed The cells of the second population (produced mainly after six weeks) will not have reached the end of their life span at that time Random destruction seems unimportant. Thus the cumulative haemolysis during the first 15 weeks cannot be smaller than the infant's blood volume at birth however it cannot be much larger either

The total haemoglobin mass in the first 2–3 days of life in the infants of the present study was calculated as follows. The data of Mollison [8] gave the relationship between venous haematocrit and red-cell mass per kg body weight and the data of Oh & Lind [9] the relationship between capillary haematocrit and venous haematocrit in infants of this age Thus the mean total haemoglobin in these infants was calculated as 45.5 g Table 4 shows the cumulative CO excretion. Assuming that four molecules of CO are produced by the destruction of one molecule of haemoglobin (=1.34 ml CO/g Hb) the corresponding cumulative haemolysis up to and including the age of 15 weeks was 40.2 g

The 'loss' of CO thus observed may have been due to many factors. A systematic analytical error giving CO estimations below the true values could have been present and cannot be excluded despite all efforts to standardize the method carefully The collection of expired air from the infants may have induced slight hypoventilation and, therefore an underestimation of the respiratory minute volume and CO excretion. This seems to be the most probable factor involved. However it may also be of interest to discuss the possibility of extrapulmonary elimination of CO Loumanmäki [6] in studies on dogs, found that up to 1 % of the body CO pool was eliminated each hour About one third was shown to be caused by oxidation of CO to $CO_2$. In order to give a maximum estimate of the total extrapulmonary CO elimination in infants 0–15 weeks old, let this figure be 1 % and assume that the mean CO-haemoglobin concentration is 0.5 %. Under these assumptions, the cumulative endogenous CO elimination could be 9–10 ml CO equivalent to about 7 g haemoglobin, which might explain the difference between observed and expected CO excretion. These estimations, however are uncertain.

The uncertain agreement between the theoretical haemoglobin catabolism and that calculated from the CO studies make the interpretation of the latter somewhat difficult. The simplest way to correct for this would be to assume that the combined errors at any moment are proportional to the observed CO excretion. The observed values could then be corrected by simple multiplication by the factor 45.5/40.2 = 1 13 However the extrapulmonary CO elimination observed by Loumanmäki is not proportional to the pulmonary CO excretion but to the total body pool of CO The underestimation of CO excretion by hypoventilation, on the other hand may well be proportional to the observed CO excretion. The relative im

portance of these two forms of under estimation of the true endogenous CO formation is difficult to evaluate and in view of this no correction at all was made

*Haemoglobin catabolism versus age.* Table 4 gives some data on haemoglobin catabolism in the different age periods studied. The daily catabolism is calculated both per kg body weight and per infant. As can be seen, during the *first week of life* infants catabolise about 185 mg haemoglobin per kg body weight. This is twice that of an adult. One and a half per cent of the total haemoglobin mass is destroyed per day. Since the red cell population at birth contains a large number of young cells, a much lower figure might be expected. As pointed out earlier no difference was found between infants showing ABO-compatibility with their mothers, and those who were ABO-incompatible. This must indicate that ABO incompatibility is not always associated with hyperhaemolysis.

There are few studies of new born infants which give results directly comparable to the present estimation of haemoglobin catabolism. Many studies of the life span of the erythrocytes of neonates have indicated shorter survival of those cells, but give little information on the haemoglobin catabolism during the first week. For a review see Zipursky [13]. The present analyses probably form the first direct estimate.

A progressive decrease of CO excretion is characteristic of the age period 1-18 weeks. Fig. 1 shows the corresponding decrease in haemoglobin destruction. No attempt will be made here to analyse this curve in detail, however some conclusions seem obvious. Firstly the curve is not

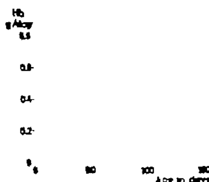



Fig. 1 Daily haemoglobin catabolism. Cf. Table 4

compatible with the assumption that foetally and neonatally formed erythrocytes behave like adult erythrocytes. If this were true much less haemoglobin would be catabolized at the beginning of the period since the red cell population in the new born must have a lower mean cell age than that in the adult. The present findings seem compatible only with a rather wide variation of the life expectancy of the erythrocytes of the new born. It is unknown whether this is due to random destruction of the infant's red cells or whether it means that cells formed during different stages of foetal life have different but definite life spans. Obviously a combination of these two possibilities may also explain the findings.

## Summary

The pulmonary carbon monoxide excretion was measured in 29 normal infants 0-18 weeks old on a total of 236 occasions. The cumulative CO excretion during the first 13 weeks of life was found to be 88% of that expected on the assumption that 4 molecules of CO are released on the breakdown of 1 molecule of haemoglobin.

The mean daily haemolysis during the

first week of life was estimated to be 185 mg haemoglobin per kg body weight, or 1½ % of the total haemoglobin mass. In these normal infants ABO incompatibility between mother and child did not increase haemolysis

Between the second and twelfth weeks of life the daily haemolysis was about 100 mg per kg. Minimal haemolysis was then observed between the 13th and 18th weeks. This indicates that the life span of most erythrocytes formed during the foetal and neonatal periods is less than 13 weeks, or about 90 days. Also the findings indicate that the behaviour of the erythrocytes formed during these periods must differ fundamentally from that of erythrocytes from adults.

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# Umbilical Cord Blood and Capillary Blood in the Evaluation of Anemia in Erythroblastosis Foetalis

by PETER JOHAN MOE

In addition to hyperbilirubinemia anemia at birth is frequently used as an indication for exchange transfusion. So far the value of cord blood in the assessment of neonatal anemia has not been compared with that of capillary blood. This has been the purpose of the present study.

In the newborn period, venous blood yields more reliable results as to hemoglobin concentration, hematocrit and the number of red blood cells than capillary blood [4, 9, 10]. Unfortunately, however, venipuncture in neonates may be difficult and traumatic.

## Material and Methods

*Umbilical cord blood* was obtained at the Maternity Unit, Department of Obstetrics and Gynecology, at this hospital from 72 healthy and 54 erythroblastotic infants. Early clamping of the cord was attempted, but this may have been delayed for about 1 minute. About 5 ml whole blood was collected in each of 2 glass tubes containing dried heparin. Samples with gross blood clots in both tubes were excluded (9 out of 135 samples).

*Capillary blood* was obtained from a 2 mm deep cut made in the medial side of an unwarmed heel with a Meda-point blood lancet in 38 of the healthy and 11 the erythroblastotic infants 20–40 minutes after delivery.

*Hemoglobin concentration, hematocrit and red cell counts* were determined in duplicate by methods reported previously [6, 7].

*Definition of anemia.* In this article the term anemia has been used in cases with hemoglobin concentration reduced below 2–3 s.d. (mean normal − 2 standard deviations). The hematocrit and red cell counts have served as a check on the hemoglobin results.

## Results

Hemoglobin obtained in cord blood from 72 healthy infants corresponded well with previous reports (Table 1). Capillary blood from 38 of the healthy infants gave higher values of hemoglobin, hematocrit and number of red blood cells than those of cord blood (Table 1).

Based on bilirubin and capillary hemoglobin level, exchange transfusion was performed in 41 of the 54 erythroblastotic infants. Of these, 25 had anemia with cord blood, whereas only 14 had capillary hemoglobin below normal (Table 2, Fig. 1). None of the remaining 11 infants with low cord hemoglobin had a capillary hemoglobin concentration of 16–19.5 g per 100 ml (Fig. 1).

TABLE 1 *Normal hemoglobin concentration in umbilical cord blood*

| Author | Year | Number of measurements | Hemoglobin concentration (g per 100 ml whole blood) Mean ($\bar{x}$) | s.d. | $\bar{x}$ − 2 s.d. |
|---|---|---|---|---|---|
| Waugh et al. [12] | 1939 | 33 | 15.36 | — | — |
| De Marsh et al. [3] | 1941 | 22 | 14.90 | 1 63 | 12.6 |
| Mollison et al. [8] | 1949 | 63 | 16.35 | 1.34 | 13.7 |
| Brody [1] | 1960 | 156 | 15.81 | 2.03 | 11.7 |
| Present study | 1964 | 72 | 15.62 | 1.37 | 12.9 |

All but one (with pronounced anemia at the age of 5 days) of these 11 patients showed an increase in serum bilirubin level to above 20 mg per 100 ml.

Normal values were found in umbilical cord blood as well as in repeated capillary blood determinations in 13 erythroblastotic infants not subjected to exchange transfusion.

## Discussion

In contrast to venous blood, cord blood is easily obtained. For assessment of erythroblastosis it is advantageous that blood samples are obtained immediately after delivery With capillary blood, significantly higher values were obtained The reason for this is not entirely obvious but placental transfusion may at least partly be responsible [2, 9] Warming of the heel prior to sampling may improve the results (unpublished data)

The fact that 25 of the 41 infants who required exchange transfusion demonstrated cord hemoglobin concentration below the observed normal range suggests a high proportion of anemia in these circumstances. In capillary blood, definite anemia was found in only 14 of the patients. Among these 54 cases of erythroblastosis, the incidence of anemia demonstrated in capillary blood was, as with earlier observations [3, 5 11], about 25 % whereas a much higher incidence was demonstrated in umbilical cord blood. To detect a moderate anemia in neonates, umbilical cord blood is therefore probably preferable In order to obtain further information, a comparison between cord, capillary and venous blood values in healthy and erythroblastotic infants is advisable.

TABLE 2 *Comparison between umbilical cord and capillary blood in 36 of the 72 healthy infants (Table 1)*

| Source | Hemoglobin (g/100 ml) Mean ($\bar{x}$) | s.d. | $\bar{x}$ − 2 s.d. | Hematocrit (Vol %) Mean ($\bar{x}$) | s.d. | $\bar{x}$ − 2 s.d. | Erythrocytes (Mill/mm³) Mean ($\bar{x}$) | s.d. | $\bar{x}$ − 2 s.d. |
|---|---|---|---|---|---|---|---|---|---|
| Capillary blood | 19.4 | 2.2 | 14.8 | 65.3 | 8.6 | 48.1 | 5 32 | 0.65 | 4 02 |
| Cord blood | 15.7 | 1 4 | 1 9 | 51 7 | 4.7 | 4[illegible] | 4 39 | 0.40 | 3 [illegible] |

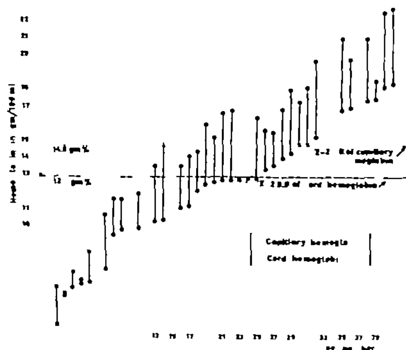


Fig 1 Umbilical cord and capillary hemoglobin values in the 41 patients who were exchange transfused.


## Summary

Figures for normal hemoglobin concentration, hematocrit and red blood cell counts in umbilical cord and capillary blood shortly after delivery are presented. In capillary blood, significantly higher values were found. In 41 erythroblastotic infants who where exchange transfused, anemia was found in cord blood of 25 With capillary blood, only 14 of the patients had anemia.

In cases suspected of erythroblastosis, heparinized cord blood should be saved.


TABLE 3 *Number of the 41 infants who were exchange transfused with demonstrable anemia*

| Source | Hemoglobin below $\bar{x} - 2$ S.D | Hematocrit below $\bar{x} - 2$ S.D. | Erythrocytes below $\bar{x} - 2$ S.D. |
|---|---|---|---|
| Capillary | 14 | 13 | 13 |
| Umb cord | 25 | 24 | 24 |

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# Respiratory Syncytial Virus Infections in Families

## *A Study of Family Members of Children Hospitalized for Acute Respiratory Disease*

*by* BO BERGLUND

In 1956 Morris and his co-workers [11] described the discovery of a new cytopathic agent from laboratory chimpanzees, subsequently called respiratory syncytial (RS) virus. Also included was a report on serologic evidence of infection by this agent in a laboratory worker who had had close contact with the infected chimpanzees. Later on, the important part played by the RS virus in causing serious lower respiratory tract diseases in infants has been fully recognized in a great number of investigations. So far however only a comparatively limited number of studies have been concerned with the incidence of RS virus infections in adults. Some of these studies will be surveyed here.

In 1960 Beem et al. [1] presented cultural and serological evidence of RS virus infection in 25-year-old person. In 1961 Kravetz et al. [10], and Johnson et al. [9] in a study on adult volunteers demonstrated infection in two thirds and mild illness in half of the subjects after intranasal administration of RS virus. As all the volunteers infected possessed detectable RS virus neutralizing antibody prior to challenge, it was concluded that these cases probably represented reinfection. In 1961 Hamre & Procknow [7] provided virus isolation and serological proof of natural infection with RS virus in 15 medical students, and described the clinical symptoms which indicated that RS virus infection in adults is associated with an upper respiratory tract disease of the common cold type. Additional information on the students RS virus infections has been published elsewhere [5, 6]. In 1962 Johnson et al. [8] in a study on military personnel provided evidence of natural RS virus infection in 22 adult subjects, and pointed out a significant difference in infection rate among those ill and controls. Recently Sommerville [12], and Carilli et al. [4] have shown that RS virus may be the most important single viral agent associated with acute exacerbation of chronic bronchitis in adults.

Little is known, however about the occurrence of RS virus infections in families, and about the intra familiar spread of these infections. The present report provides information on this subject, calling particular attention to the RS virus infection rates of adults

This study was supported by the National Research Council for Medical Sciences.

## Material and Method

### *Subjects*

In another paper [3] a report was given on the incidence of RS virus infections among 60 children hospitalized for acute respiratory

disease during an outbreak of illness associated with this virus in Turku, Finland extending from early April to late June, 1965. These individuals, who for the purpose of the present study have been designated as index children, are not included in this series, but the virologic examinations carried out on them form the basis for the division of their family members—who will constitute the study subjects—into different groups. Index children with one or more positive virus isolations and/or a 4-fold or greater increase in the RS virus complement-fixing (CF) antibody titer were diagnosed as having RS virus infection. Index children with negative RS virus isolations and an RS virus CF antibody titer of <1/4 in their paired serum specimens were diagnosed as having no RS virus infection. Negative RS virus isolations, but reciprocal RS virus CF antibody titers of 32–32, 16–16, and 64–32, respectively (in three cases) indicated that the index child had questionable RS virus infection.

The present study series consists of the family members of the index children, including mothers, fathers, brothers and sisters, as well as 4 grandmothers and a few young adult household helps or family associates living together permanently in the same flat. Brothers and sisters were regarded as being children, other family members as adults. The basis for the division of the series into adults and children was a natural one, and it caused only three 16-year-old and three 15-year-old subjects to be designated as children. The remainder of the children were 13 years or under. The adults were 18 years or over.

The subjects were divided into 3 groups: Family members of children with RS virus infection (Group 1), family members of children with no RS virus infection (Group 2—controls), and family members of children with questionable RS virus infection (Group 3). Only Groups 1 and 2 will be examined in greater detail.

Group 1 was made up of 25 families, consisting of 46 adults whose mean age was 29 and of 29 children whose mean age was 7 years. Group 2 was made up of 23 families consisting of 44 adults whose mean age was 28 and of 13 children whose mean age was 5 years. Group 3 was made up of 3 families.

The study was carried out in May and June, 1965.

*Collection of material and clinical information*

When a child was admitted to the ward, its family members were requested to visit the out-patient clinic of the hospital within 3 days for blood sampling. On this occasion they were given questionnaires to be filled in and returned at the 2nd blood sampling 3–7 weeks afterwards. The questionnaires were intended to provide information concerning the occurrence of symptoms, such as nasal discharge, cough, hoarseness, sore throat, and the date of onset of these symptoms. Adults who developed respiratory symptoms in the period between the first and second blood sampling returned to the out-patient clinic within 2 days for the collection of 2 single throat swabs for virus isolation. The isolation technique has been described elsewhere [3].

*Determination of CF antibodies in serum specimens*

The CF antibody titration was performed according to a microtechnique described previously [3]. Antigens of the following agents were employed in the tests: RS virus, influenza A, B, parainfluenza 1, adeno- and cytomegalovirus.

*Determination of RS virus neutralizing antibodies in serum specimens*

Only adult sera were tested. Cell cultures of a continuous line of human amnion (U cells) were used for preparation of the virus. The growth and maintenance media of these cells have been described previously [3]. Fresh maintenance medium was used as virus and serum diluent in all tests. The virus (Randall strain) was centrifuged at 2500 r.p.m. for 15 minutes and diluted to contain approximately 100 $TCD_{50}$ units of virus. The sera were inactivated at +56°C for 30

TABLE 1 *RS virus CF antibody titer rises in family member of children hospitalized for acute respiratory disease*

| Reciprocal rise in RS virus CF antibody titer | Group 1 Family members of children who had RS virus infection[a] | | Group 2 Family members of children who had respiratory infection not due t RS virus[b] | | Group 3 Family members of children with quest onable RS virus infection[c] | |
|---|---|---|---|---|---|---|
| | N adults | No. brothers and sisters | No. adults | No. brothers and sisters | N adults | No. brothers and sisters |
| <4–4 | 11 | 2 | 5 | — | — | — |
| 4–8 | 1 | | 1 | | | |
| 4–16 | 1 | 1 | | | | |
| <4–8 | 4 | | | | | |
| <4–16 | | 1 | | | | |
| <4–32 | | 2 | | | | |
| <4–64 | | | | | | |
| No. family members tested | 43 | 26 | 37 | 11 | 6 | 1 |

[a] RS virus isolation from throat swabs, and/or 4-fold or greater rise in CF antibody titer
[b] No RS virus isolated, CF titer <1/4 in all sera examined.
[c] No RS virus isolated, CF titer of acute serum 1/16, 1/32 or 1/64, no rise in titer

minutes and used in 4 fold dilutions of 1/4 1/16, 1/64 and 1/256. Equal volumes of diluted serum and virus suspension were mixed, the mixture incubated at room temperature for 1 hour and used for inoculating the cell cultures, 0.2 ml/tube and 3 tubes/serum dilution. Cell and serum controls were included in each test. The tests were read after 2–4 days when simultaneous titrations indicated the presence of at least 150 $TCD_{50}$ units of virus/0.1 ml of virus dilution used in the test.

### *Statistics*

Data were tested for significance by means of the $\chi^2$ test, or—if the number of subjects was too small t permit the use of this—by means of the following formula:

$$\text{Prob.} = \frac{( \quad b)!( \; +d)!( \; +c)!(b+d)!}{N! \; !b! \; !d!}$$

where a, b, and d are cell frequencies and N is the total number of subjects. The risk included in rejecting the null hypothesis p 2 Prob

## Results

Table 1 shows the number of adults and children who developed increases in CF antibody to RS virus. In Group 1 4-fold or greater (diagnostic) rises in titer were observed in 7 adults of 43 (16 %) whereas diagnostic titer rises did not occur at all among the 37 adult subjects in Group … The difference is statistically significant (Prob = 0.0101 $p < 0.05$) If all CF antibody increases of the adults are considered (including also the rises from <1/4 to 1/4 and from 1/4 to 1/8) and if any rise in titer is taken to indicate an association with recent or present RS virus infection this association rate is 19/43 (44 %) in Group 1 and 6/37 (16 ) in Group 2, and this difference is also statistically significant ($\chi^2 = 0.00$ $p < 0.0?$)

Moreover Table 1 shows that 6 (23 %) f the 26 children in Group 1 developed diagnosti titer rises to RS virus and that

TABLE 2. *RS virus CF antibody titers in family members of children hospitalized for acute respiratory disease. Family members with rise in titer are disregarded.*

| Reciprocal RS virus CF antibody titer in 1st serum | Group 1 Family members of children who had RS virus infection[a] | | Group 2 Family members of children who had respiratory infection not due to RS virus[b] | | Group 3 Family members of children with questionable RS virus infection[c] | |
|---|---|---|---|---|---|---|
| | N adults | No. brothers and sisters | No. adults | No. brothers and sisters | No. adults | No. brothers and sisters |
| < 4 | 17* | 9 | 29*** | 12* | 2 | 1 |
| 4 | 4 | 2 | 5** | | 3 | 1 |
| 8 | 4 | | 2* | | 2 | |
| 16 | 1 | 3 | 1 | | | |
| 32 | | | 1 | | | 1 |
| 64 | 1 | 3 | | 1 | | |
| 128 | | 1 | | | | |
| > 256 | | 3 | | | | |
| N family members tested | 27 | 21 | 38 | 13 | 7 | 3 |

[a] RS virus isolation from throat swabs, and/or 4-fold or greater rise in CF antibody titer
[b] No RS virus isolated. CF titer < 1/4 in all sera examined.
[c] RS virus isolated. CF titer of acute serum 1/16, 1/32 or 1/64; no rise in titer
Asterisked numbers include subjects (one asterisk designates one subject) with only one examined serum specimen.

antibody increases were not observed in any of the 11 children in Group 2. As the number of children in Group 2 is however small the difference possibly for this reason, is statistically insignificant (Prob = 0.099). If all CF antibody increases in the children are considered (including also the rises from < 1/4 to 1/4 and from 1/4 to 1/8) the number of seropositive children in Group 1 amounts to 8 out of 26 (31 %) and in Group 2 none out of 11. This difference also remains statistically insignificant (Prob = 0.0405).

Table 2 shows the 1st serum RS virus CF antibody titer of all those adults and children who did not develop any RS virus CF antibody increase at all in their paired specimens, or from whom a 2nd blood specimen was not taken. In Group 1 10 adults out of 27 (37 %) had antibody to a titer of 1/4 or higher in Group 2 nine adults out of 38 ( 4 %). The difference is statistically insignificant ($\chi^2 = 0.8$). If the children shown in Table 2 are considered, however the incidence of titers of 1/4 or higher was statistically significantly more frequent in Group 1 (12/21 = 57 %) than in Group (1/13 = 8 %). Prob = 0.004 $p < 0.01$.

If the children in Tables 1 and  are considered together and if a rise in titer or a titer of 1/4 or higher is taken to indicate an association with recent or present RS virus infection, the association rate is then 20/29 (69 %) in Group 1 and only 1/13 (8 %) in Group [illegible] This difference is statistically highly significant ($\chi^2 = 11.14$, $p < 0.001$).

Table 3 provides detailed information

Table 3. *Fourfold or greater RS virus CF and/or neutralizing antibody titer rises 1) in parents of children hospitalized with RS virus infection 2) in parents of children hospitalized with respiratory infection not due to RS virus*

| Group of parents | Parents no. | Sex | Age in years | Time in weeks between 1st and 2nd blood sampling | Reciprocal CF titer 1st serum | Reciprocal CF titer 2nd serum | Reciprocal neutralizing titer 1st serum | Reciprocal neutralizing titer 2nd serum | Respiratory symptoms starting between 1st and 2nd blood sampling |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | F | 31 | 5 | <4 | 8 | 4 | 16 | Rhinitis |
| | 2 | F | 30 | 5 | <4 | 8 | 4 | 4 | Sore throat |
| | 3 | F | 23 | 5 | <4 | 8 | 4 | 16 | Sore throat |
| | 4 | M | 25 | 5 | <4 | 16 | 4 | 16 | — |
| | 5 | F | 28 | 4 | <4 | 16 | 4 | 16 | Rhinitis |
| | 6 | F | 19 | 5 | <4 | 8 | <4 | 4 | — |
| | 7 | M | 23 | 7 | <4 | 16 | 4 | 16 | — |
| | 8 | F | 32 | 6 | <4 | 4 | 4 | 16 | Rhinitis, cough |
| | 9 | F | 30 | 5 | <4 | 4 | 16 | 64 | Rhinitis, cough |
| | 10 | F | 28 | 5 | <4 | 4 | 4 | 16 | Rhinitis, hoarseness, sore throat |
| 2[b] | 11 | M | 23 | 5 | <4 | 4 | 64 | 256 | — |
| | 12 | F | 24 | 6 | <4 | <4 | 16 | 64 | — |

Paired sera of 43 adults tested.
Paired sera of 37 adults tested.
Parents nos 3 and 4 wife and husband.

on all those adults (=parents) who responded with a diagnostic increase in CF and/or neutralizing antibody to RS virus. It is noticeable that the majority were females. As already shown above diagnostic CF antibody rises were detected in 7 adults in Group 1 and in none of the adults in Group 2. As shown in the Table the adult RS virus CF antibody responses were surprisingly weak, as the titers in any case did not exceed 1/16. Of the 7 CF positive parents, 5 also exhibited a 4-fold rise in the RS virus neutralizing antibody titer and of the 10 neutralization positive parents, 5 displayed 4-fold or greater rise in the CF antibody titer The RS virus associated symptoms were suggestive of a mild upper respiratory tract disease similar to that described by others [7 10 11]. Of the 7 CF positive parents, separately considered, 3 individuals, two of whom were male and one female did not develop respiratory symptoms.

Table 4 demonstrates the occurrence of adenovirus infection in a boy and his mother followed by RS virus infection this also affecting the father

At the time of the collection of the serum specimens 3–4 months had passed since an outbreak of respiratory illness due to influenza A2 virus in February 1965, involving the whole city and extensive areas of the country Examination of the 1st serum from all the 90 adults of this study and from 7 additional adults of the same age from whom blood specimens were collected at the same time indicated that the reciprocal influenza A CF antibody

TABLE 4 *Simultaneous adenovirus infections followed by simultaneous RS virus infections in members of a small family*

| Family member | Age in years | Dat of blood sampling | Reciprocal adenovirus CF titer | Reciprocal RS virus titer | |
|---|---|---|---|---|---|
| | | | | CF | Neutralization |
| Son | 11/12 | 31.5 | 4 | <4 | <4 |
| | | 11.6 | 32 | <4 | <4 |
| | | 29.7 | 16 | 32 | 16 |
| Mother | 23 | 1.6 | 4 | <4 | 4 |
| | | 22.6 | 16 | <4 | 4 |
| | | 29.7 | 8 | 8 | 16 |
| Father | 25 | 1.6 | 16 | <4 | 4 |
| | | 22.6 | 16 | <4 | 4 |
| | | 29.7 | 16 | 16 | 16 |

Treated in hospital for an infection (Bronchitis ac., Tonsillitis) which started on May 28, and for another (Bronchopneumonia l.a., Otitis media l.a.) which started on June 19. Adenovirus was isolated from throat swabs on May 31 and RS virus on June 24.

titer was 128 in 6 %, 64 in 30 %, 32 in 30 %, 16 in 14 % and ≤8 in 20 % of the subjects tested. The adult CF antibody titers were thus in general considerably higher with influenza A than with RS virus. No influenza A virus CF antibody increases were detected.

Table 5 shows separately the number of adult subjects developing symptoms of acut respiratory tract illness in Groups 1 and 2 at various intervals during the study. At each interval the difference between the illness rates of the two groups was statistically of no significance. As seen in the table 17 adults out of 46 (37 %) in Group 1 and 18 out of 44 (41 %) in Group 2 had respiratory symptoms in the period between the first and second blood sampling ($\chi^2 = 0.03$) and 11/46 (24 %) in Group 1 and 5/44 (11 %) in Group 2 immediately before the 1st blood sampling ($\chi^2 = 1.64$). The number of adults developing respiratory symptoms at either or both time intervals was 28/46 (61 %) in Group 1 and 23/44 (52 %) in Group 2 ($\chi^2 = 0.37$). If also the children were considered, together with the adults, the number of subjects showing respiratory symptoms at either or both time intervals was 47/74 (64 %) in Group 1 and 31/54 (58 %) in Group 2 and the difference statistically insignificant ($\chi^2 = 0.27$).

The serological investigations for the detection of infection with agents other than RS virus were incomplete in this study as only a limited number of different viral antigens were employed in the CF tests. Therefore the causative microorganism of most of the respiratory diseases, which were apparently not due to RS virus, remained undefined. Only one adeno-, one cytomegalo- and one parainfluenza 1 virus infection was observed in the children, and only 1 adenovirus infection in the adult subjects (the latter is recorded in Table 4).

Throat swabs were obtained only from a total of 16 subjects (adults) two of whom

TABLE 5 *Incidence of acute respiratory symptoms in adult family members of children hospitalized for acute respiratory disease*

| | Group 1 | | Group 2 | |
|---|---|---|---|---|
| | Adult family members of children who had RS virus infection | | Adult family members of children who had respiratory infection not due t RS virus | |
| Time-localization of onset of symptoms | No. of adult family members | % | No. of adult family members | % |
| Between 1st and 2nd blood sampling | 17 | 37 | 18 | 41 |
| During 3-week period preceding 1st blood sampling | 11 | 24 | 5 | 11 |
| Symptoms lacking | 18 | 39 | 1 | 48 |
| Total | 46 | | 44 | |

Rhinitis, cough, hoarseness and/or sore throat.
RS virus isolation from throat swabs, and/or 4-fold or greater rise in CF antibody titer
No RS virus isolated, CF antibody titer <1/4 in all sera examined.

subsequently developed a diagnostic antibody response to RS virus. No virus was isolated from any subject

## Discussion

*Laboratory diagnostic aspects of RS virus infection in adult*

The CF test did not reveal proof of infection with RS virus in a control group of adult family members of children free from RS virus infection, but provided evidence for actual infection with this virus in 16 % of the adult family members of children with confirmed RS virus infection. As virus isolation attempts were carried out only to limited extent it is clear that this percentage only represents minimal estimate of the infection rate

It is well known that dult may harbour RS virus in their throat secretions, indicating infection, but without developing a serologic response t lt [ 0]. Under these circumstances it seems plausible that different adult individuals would respond to their RS virus infection differently and that slight increases of antibody would also be encountered frequently In the present study in cases of RS virus CF antibody increases the titer never exceeded 1/16, which is noticeable nd supports this assumption. Another explanation for the low titers could be that the convalescent sera were possibly collected at an unsuitable time .g before the occurrence of a maximal response Johnson *et al* [9] have shown that the CF titer of adult volunteers did not reach its maximum until 14–18 days after challenge with virus In any event the diagnostic adult CF antibody responses in the present study were apparently not due to laboratory error a similar responses did not occur at ll mong the adult of the control group

Although the series is too small to allow conclusions the findings suggest that cer

tain similarities may exist between adults and young infants with respect to the extent of their RS virus CF antibody response In the light of the results of the present study it would seem desirable for the detection of RS virus infection, to start the CF titration of adults sera from a dilution as low as possible preferably 1/4 or 1/2.

The adult CF antibody titers were throughout considerably lower to RS than to influenza A virus. The sera were collected during the second half and the end of the RS virus outbreak, 3–4 months after an outbreak due to influenza A virus. The findings seem to suggest, either that the adults were more extensively infected with the influenza virus, or that their CF antibody response to infection with this virus was stronger than to infection with RS virus.

The results of the determination of the serum RS virus neutralizing antibody titer obtained in the present study cannot be more carefully evaluated. Fourfold instead of 2 fold serum dilutions were employed and this was probably likely to diminish the reliability of the test to some extent. Johnson *et al.* [9] in their study on experimentally infected human adult volunteers, however observed that the neutralization and the CF tests are about equally sensitive as means of detecting RS virus infection, but that the availability of the neutralization test may be limited in cases where the acute serum already contains neutralizing antibody to a high titer

*Epidemiological aspects of intra familiar spread of RS virus infection*

By comparing the CF antibody titers in family members of children with confirmed RS virus infection and the CF titers of family members of children with other respiratory infections, it was found that present or recent RS virus infections occurred to a higher frequency in the former group than in the latter and that the difference was significantly higher than expected on a random basis. It can thus be *concluded that RS virus infections spread* to a considerable extent within families, a substantial proportion of the children as well as the adult family members contracting infection. This family-occurrence seems to be one of the epidemiological characteristics of RS virus infection.

The parents displayed rising titers, whereas the titers of the children were either rising or had already reached their maximum. It seems likely that one or more of the children of the families first caught the infection from somewhere outside the home, these children then infecting their brothers and sisters and the parents. In the light of the results, it appears probable that RS virus was not brought to the family by the adult members of it but by the children. The infection and illness rates among the mothers were higher than those among the fathers. As the mothers presumably had a more intimate contact with the children than the fathers, this seems to suggest that virus dosage and exposure time may play a part in the development of RS virus infection in adults.

## Summary

The present report describes the frequency of RS virus infections in 51 families at a time when this virus was prevalent in the community Index children, one

child from each family hospitalized for acute respiratory disease, constituted the basis for the selection of these families for study The index children themselves, and the results of the virological examinations carried out on them, were not included in this study but have been accounted for previously [3]. The study group (Group 1) was made up of family members of index children with RS virus infection, the control group (Group ) of family members of index children with respiratory disease due to agents other than RS virus. Paired sera were examined for CF and neutralizing antibody to RS virus. In evaluating the results only those results obtained with the CF technique were considered in greater detail.

The adult CF antibody response to RS virus infection was always weak, as compared to that of most of the children Diagnostically rising (4-fold or greater antibody increase) titers were observed in 16 % of the parents of the study group and not at all in the control parents ($p < 0.05$). When adults with no rise in RS virus titer were considered separately the titers were found on average to be of the same magnitude within both groups.

The frequency of symptoms of respiratory disease was a little although not significantly higher among the adults of the study group (61 %) than among the adult controls (.. %).

Diagnostically rising CF titers were noticed in 23 % of the children of the study group, but not at ll among the control children. The difference was statistically insignificant When children with no rise in titer were considered separately it was found that 57 % of the children of the study group and only 8 % of the control children had CF antibody to a titer of 1/4 or higher ($p < 0.01$). If a diagnostically rising titer or a CF antibody titer of 1/4 or higher was taken to indicate an association with recent or present RS virus infection, this association rate was 69 % among the children of the study group and only 8 % among the control children ($p < 0.001$)

On the basis of the results it is concluded that RS virus infection spreads in families involving a considerable number of the children, but lso sometimes either or both of the parents. In general the infection appeared to have been brought into the families by the children who later on infected their parents. The infection and illness rates were higher among the mothers than among the fathers

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# Rubella Immunity of Pregnant Women in Stockholm

by ARNE SVEDMYR and CLAES THORÉN

Knowledge of the incidence of susceptibility to rubella in females of fertile age is essential for estimation of the risk for maternal infection during pregnancy and for evaluation of the need for specific prophylaxis. Pregnant women in different parts of USA [6] and Great Britain [1 2] as well as in Eskilstuna Sweden [3] are reported to lack rubella neutralizing (NT) antibody in 5–20% and this incidence is apparently considerably higher in Hawaii [5 6] and southern Japan [4]

In this study the frequency of rubella NT antibody was determined in two groups of pregnant women in the city of Stockholm those applying for gamma globulin prophylaxis due to exposure t rubella as well as a normal control group

## Materials and Methods

In Sweden women who do not know if they have previously had rubella are allowed free gamma globulin if exposed to rubella in the first trimester of pregnancy The public gamma globulin prophylaxis in Stockholm is centralized t Crown Princess Lovisa Children Hospital. Since April 1963 a blood specimen for a rubella NT test was collected from these women before administration of gamma globulin. Between the day of exposure and that of the bleeding mean of 3.6 ± 1 7 days elapsed. In a single case the interval exceeded 9 days; actually this case happened to be seronegative Even if the first contact might have happened a week earlier than known to the patient possible infection should therefore hardly yet have resulted in a positive NT test

As a control group pregnant women were randomly selected, regardless of history of rubella or exposure from the same Maternity Welfare Centers from which the prophylactically treated cases were referred. Blood specimens were obtained from three consecutive pregnant women for each prophylaxis case until bout 100 were collected.

Until November 1966, 54 women of the prophylaxis group and 104 controls have been examined. The mean age for the groups were 27.8 and 26.1 years respectively the corresponding medians 27 and 25. The difference between the means is not significant (Student s t-test).

The NT tests were performed in RK 13 cells as described in detail in he before mentioned paper in this journal (3)

## Results

The results are summarized in Table 1 The frequency of NT antibody to rubella virus was the same among pregnant women applying for gamma globulin prophylaxis as in the control group, 87 %. Within the control group on the other hand all

TABLE 1 *Rubella NT Antibody in Pregnant Women in Stockholm*

| Material | Age in years | | N of cases | Cases with antibody |
|---|---|---|---|---|
| | median | range | | |
| Women applying for prophylaxis | 27 | 18–44 | 54 | 47 |
| Pregnant controls | 25 | 17–41 | 104 | 90 |

38 individuals who reported that they had undergone rubella also had NT antibody whereas 52 (79 %) out of the 66 without rubella history were seroimmune This difference is significant ($p < 0.01$ $\chi^2$ test)

In the control group the mean age of the seropositive cases with a history of rubella was 25.5 years, of those without knowledge of previous rubella 26.4 years. The seronegative cases had a mean age of 26.4 years.

## Discussion

The frequency of rubella NT antibody among the pregnant women investigated, 87 % is well in accordance with the 83 % seropositivity of 35 cord blood specimens collected in another urban area, Eskilstuna, not far from Stockholm [3] In the latter report 82 % of 175 women aged 15 or more had rubella NT antibody In neither of these areas had there been any large epidemic of rubella since the years of 1951 and 1956 [3] As mentioned above a similar incidence of immunity has been found in the last years in U.S.A. [6] as well as in Great Britain [1] Some variation in immunity between various parts of a country must be expected, however as illustrated by the 80–95 % range recently found in Great Britain [2].

The association between history of overt rubella and seroimmunity that was most evident in the younger age groups of the Eskilstuna study [3] was obvious also in the control group of normal pregnant women in Stockholm. In the prophylaxis group lack of rubella history was a prerequisite for obtaining free gamma globulin, consequently such information may be suspected to be biased. Yet the incidence of seroimmunity of the prophylaxis cases was not significantly different from that of the random controls without history of rubella, and taken together these groups had a significantly lower incidence of antibody than had subjects with rubella history ($p < 0.01$)

## Summary

The frequency of rubella NT antibody in pregnant women from Stockholm has been examined in 54 cases applying for immune prophylaxis after exposure to rubella as well as in 104 normal controls. In both groups about 90 % were seroimmune Seroimmunity was more common among subjects with a history of rubella

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REVIEW ARTICLE

# Progressive Spinal Muscular Atrophy with Onset in Infancy or Early Childhood

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Cases of progressive spinal muscular atrophy starting in early childhood were first described by Werdnig [55 56] and Hoffmann [24 25 26 27] Both described patients who appeared healthy during the greater part of infancy Towards the end of the first year slowly progressive weakness and hypotonia appeared, starting in the pelvic and hip muscles. The children died from respiratory complications between 3 and 7 years of age. At autopsy the anterior horn cells were found to be partly destroyed and reduced in number

A few years later Oppenheim [41 42 43] reported children, who in early infancy perhaps already at birth had generalized muscular hypotonia with weakness and absence of muscle reflexes. He reported no follow up or results of histopathologic studies but claimed that his cases were non familial and tended to improve He ascribed the condition to delayed maturation of the muscles. For several years Werdnig Hoffmann s and Oppenheim s diseases were considered separate diseases differing in age at onset and prognosis

However already at the beginning of the century patients were reported in whom familial incidence was combined with neonatal onset of progressive symptoms [5 11 32] Extensive reviews of infants with weakness and hypotonia have established that one cause of this syndrome is progressive spinal muscular atrophy with early onset and poor prognosis [6 54] The term Werdnig Hoffmann s disease is now used both for the cases with neonatal onset and for those with onset during the second half year and the conditions are considered the same disease

Slowly progressive spinal muscular atrophy with onset of symptoms between 3 and 9 years of age was described by Wohlfart *et al* [59] and Kugelberg & Welander [30] The authors considered this juvenile type an independent disease distinguishable from Werdnig Hoffmann s disease by its later onset slower progress and lack of bulbar symptoms They also stressed absence of pyramidal tract signs differentiating the condition from amyotrophic lateral sclerosis.

However several patients have been described with a clinical picture apparently representing a transitional form between the infantile and the juvenile types of progressive spinal muscular atrophy (6, 8,

TABLE 1 *Symptoms and course in 25 children with progressive spinal muscular atrophy*

| Case no. | Type | Sex | Affected sibs/total no. of sibs | Age at onset years | Age at first exam. years | Age at latest exam. years | Age at death years | First noticed symptoms |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | F | 0/0 | 2/1 | 4/12 | | 6/12 | Generalized weakness inability to lift head |
| 2 | 1 | F | 0/0 | Newborn | 3/12 | | 8/12 | Generalized weakness, inability to lift head |
| 3 | 1 | F | 2/3 | 2/12 | 6 12 | | 8/12 | Generalized weakness, inability t lift head |
| 4 | 1 | M | 1/1 | 2 3/12 | 7/12 | | 1 | Generalized weakness, inability t lift head |
| 5 | 1 | M | 1/1 | 3 12 | 5/12 | 1 5/12 | Alive | Inability to lift head, poor kicking |
| 6 | 1 | M | 0/3 | Newborn | 1 | | 3 | Generalized weakness, inability t lift head |
| 7 | 1 | F | 0/0 | 8/12 | 8/12 | 2 | Alive | Generalized weakness, inability to lift head |
| 8 | 1 | M | 0/1 | 11 (probably earlier) | 1 4/1.. | 1/2 | Alive | Generalized weakness, inability to lift head |
| 9 | 1 | M | 1/1 | Newborn | 12 | 13 | Alive | Generalized weakness inability to lift head |
| 10 | 2 | M | 0/1 | 8–10/12 | | | Alive | Weak legs, could not get up |
| 11 | 2 | F | 0/1 | 2/1 | 2 1/2 | 8 | Aliv | Weak kicking thin thighs could not get up |
| 12 | | F | 0 1 | 1 | 12 | 14 | Alive | Waddling gait hyper extended knees, could not get up |
| 13 | | F | 0/0 | 1 1/* | 2 4/12 | 3 1/2 | Alive | Did not walk unaided, could not get up |
| 14 | 2 | M | 0/2 Father affected | 2–3/12 | 3 1/2 | 4 1/2 | Alive | Waddling gait thin thighs |
| 15 | | F | 0/1 | 2 | 3 1/* | 4 1/2 | Aliv | Waddling gait could not get up |
| 16 | 2 | F | 0/2 | 3/12 | 14 | 15 | Alive | Did not walk unaided, could not get up |
| 17 | | M | 0/0 | 10/12 | 4 1/2 | – | Alive | Did not walk, could not get up |
| 18 | | F | 0/1 | 1 1/* 2 | 9 | 14 1/* | Alive | Waddling gait could not get up thin thighs |
| 19 | 2 | M | 1/1 | 10/12 | 11 | | Alive | Could not get up thin thighs |
| 20 | 3 | F | 1/2 | 3/12 | 8 1/2 | 7 1/2 | Alive | Waddling gai could not get up, thin thighs |
| 21 | 2 | M | 1/2 | Newborn | 1 1/2 | 2 1 | Alive | Thin thighs abnormall weak kicking |
| 22 | 2 | F | 0 1 | 1 | 1/2 | | Alive | Could not get up not w lk without support |
| 23 | 2 | M | 0/1 | 8–10 12 | 6 1/2 | – | Ali | Could not get up did not kick normally lost abili to sit without support |
| 24 | | F | 0/* | 1 1/* | 11 1/2 | | Aliv | Waddling gait, fell often, had to climb to get up |
| 25 | 3 | M | 0 1 | 1 1/2 2 | 16 | 20 | Al | Waddling ga could not run, difficulties in climbing stairs |

TABLE 2 *Findings in 25 children with progressive spinal muscular atrophy*

| Case no. | Type | Reflexes | Bulbar signs | Deformities, contractures | Serum enzymes | Conduction velocity | Histopathology |
|---|---|---|---|---|---|---|---|
| 1 | 1 | Absent | + | Thoracic | LDH 430, isoenzyme 1 increased GOT normal | 3 slightly low one borderline | *Neurogen atrophy* |
| 2 | 1 | Absent | – | Thoracic | LDH 400 isoenzyme 1 increased, GOT and CK normal | 4 slightly low one borderline | Neurogen trophy |
| 3 | 1 | Absent | + | Thoracic | LDH 500, isoenzyme 1 increased, GOT normal | 2 slightly low 2 borderline | *Only fat, no muscle f bres* |
| 4 | 1 | Absent | + | Thoracic | LDH 480, GOT normal | 2 borderline | Neurogen atrophy |
| 5 | 1 | Absent | + | Thoracic | LDH 570 GOT and CK normal | Normal | Neurogen atrophy |
| 6 | 1 | Absent | + | Thoracic, kypho-scoliosis | LDH 365, GOT normal | Normal | Neurogen atrophy |
| 7 | 1 | Absent | – | None | LDH 490 isoenzyme 1 increased, GOT and CK normal | N rmal | Neurogen trophy |
| 8 | 1 | Absent | + | Hips, knees | LDH 590 isoenzyme 1 increased, GOT normal CK increased (96) | Normal | Neurogen atrophy |
| 9 | 1 | Absent | – | Kyphoscoliosis, hips, knees | CK normal (48) | Normal | Neurogen trophy + *myopathic changes* |
| 10 | 2 | Absent | – | Hips, knees | N t done | Normal | Neurogen atrophy |
| 11 | 2 | Hypoactive | | None | LDH 440 GOT normal | Normal | Neurogen atrophy |
| 12 | 2 | Absent | – | Slight in hips and elbows | LDH 355 even increase of first 3 isoenzymes, GOT normal, CK increased (137) | Normal | Neurogen atrophy + myopathic changes |
| 13 | 2 | Hypoactive | – | None | LDH 440, isoenzyme 1 increased GOT and CK normal | Normal | Normal |
| 14 | 2 | Normal | – | None | Normal | Normal | Not judgeabl |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 | 2 | Hypoactiv | | None | GOT normal | Normal | Neurogen atrophy |
| 16 | 2 | Absent | | Kyphoscoliosis, hips, knees | Normal | One slightly low the rest normal | Neurogen atrophy + myopathic changes |
| 17 | 2 | Absent | | Hips, knees | Normal | Normal | Neurogen atrophy |
| 18 | 2 | Absent | + | Kyphoscoliosis, hips, knees | Normal | Normal | Neurogen atrophy |
| 19 | 2 | Hypoactive | | None | CK increased (140), GOT and LDH normal | Normal | Neurogen atrophy |
| 20 | 2 | Absent | | None | Not done | Normal | Not done |
| 21 | 2 | Hypoactive | | None | Not done | Normal | Not done |
| 22 | 2 | Absent | | None | LDH 340, isoenzyme 1 increased, GOT and CK normal | Normal | Neurogen atrophy |
| 23 | 2 | Absent | + | Kyphoscoliosis, hips, knees | Normal | One borderline, one slightly low | Neurogen atrophy + myopathic changes |
| 24 | 2 | Hypoactive | | None | Normal | Normal | Neurogen atrophy |
| 25 | 2 | Brisk, Babinski sign present | – | None | LDH 400 | Normal | Neurogen atrophy |

Electromyography showed denervation in all 25 patients.

0 16 19 20, 28 34] The present material, which includes several examples of the transitional form, illustrates the variability of the clinical picture in progressive spinal muscular atrophy with early onset. The patients are classified according to the site of the initial symptoms rather than to the age at onset in an attempt to find the early symptoms which may predict the further course

## Material and Methods

The material consisted of 25 children, 1 boys and 13 girls, in whom progressive spinal muscular trophy was diagnosed in 1961–66. The patients were 4 months t 16 years of age when first seen at the Department of Pediatrics, Lund (T bles). The diagnosis was basically made on the linical picture In addition various special studies were performed: examination of the spinal fluid, determination of the activity of serum transaminases, lactic deh drogenase [60], including separation in isoenzymes [21] and creatine kinase (cystein activated CK was determined by a method developed by Sjövall, according to the principles of Oliver [40]) The conduction velocity of the ulnar median and peroneal nerves was measured bilaterally [17 23]. Electromyography was performed on at least one arm muscle and one contralateral leg muscle A biopsy specimen, removed from the anterior tibial muscle, was examined histologically

## Results

*Inheritance* No cases were known in the patients families except those in parents and sibs The material included 3 pairs of affected sibs One child (case 5) was known to have had 2 older sibs who had died from the disease in infancy One patient (case 14) father who had had weakness of the legs since school-age was xamined clinically and electromyo-

graphically and found to have moderate weakness and wasting of the hip and thigh muscles and possibly also of the shoulder muscles. Electromyography showed signs of denervation, the conduction velocity of peripheral nerves was normal. Inquiry into the family histories of the remaining patients was non-contributory No consanguinity could be traced between parents and no blood relationship between the 22 families

*Clinical picture, course and prognosis* The age at onset was definitely below 3 years in all the patients. Because of the insidious nature of the disease, many parents were unable to date the onset Retrospectively it appeared likely that the symptoms had invariably started within the first 18 months of life

When classified according to the initial symptoms and the findings at examination, the cases fell into 3 clinical types. Type I was characterized by early and severe weakness of neck, trunk and proximal arm and leg muscles; type II by weakness initially confined to pelvic girdle and proximal leg muscles; and type III by pyramidal tract signs.

Type I. This group consisted of 9 patients including 6 in whom the first symptom noticed by the mother was the inability of the child, when prone to lift its head at the age of 2–3 months. Routine check up of 2 other children (cases 7 and 8) poorly observed by their parents revealed at 5 months and 1 year respectively general weakness and no head control. Only one mother stated that her child (case 5) had had no symptoms before age 3 months, when she noticed that his leg movements grew weaker and he became unable to lift his head.

Common to these 9 children was *the early and severe involvement of the neck and trunk muscles* which often caused deformities of the chest (Fig 1). None of the children could ever lift its head in the supine position, sit up without help or stand. Besides, they all had severe weakness of the proximal arm and leg muscles and only one (case 7) could lift the elbows or knees from the bed. Muscle reflexes were absent in all Fasciculations of the tongue were observed in 6 (cases 1 3 4 5 6 8)

Four children died in the first and one in the fourth year of life Four are still alive aged 1½ 2, 2½ and 13 years. The youngest has a severely deformed chest He can not sit without support. The 2 children in their third year have no thoracic or spinal deformities. If helped to sit up they can sit without support (Fig 2)

The oldest child (case 9) was examined for the first time at 12 years. He could never lift his head. If helped to sit up, he could sit without support from the age of 7–8 months. At age 5–7 years he developed kyphoscoliosis and was then no longer able to sit without support. Since then his condition has remained unchanged (Fig 3) This boy's younger brother is reported among the type II patients (Fig 4)

Fifteen patients belonged to type II The initial and dominating symptoms were *unusually thin thighs weakness of legs and an abnormal gait* Thin thigh muscles and abnormally weak kicking were definitely observed in one child (case 21) already during its first 3 months of life The same age of onset appeared likely in 4 other children (cases 11 14



Fig. 1 Case 2 Age 4 months.

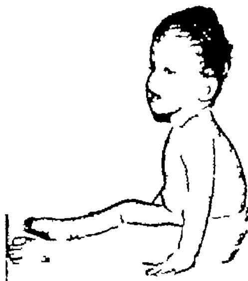


Fig. 2. Case 8 Age [illegible] years.



Fig. 3 Case 9 Age 1 [illegible] years

16–20) although their parents gave less positive information. In the remaining 10 children the first symptoms were observed when they were between the ages of 8 and [illegible]4 months.

All the patients of type II achieved early head control and could sit without support at 6–9 months. All of them also

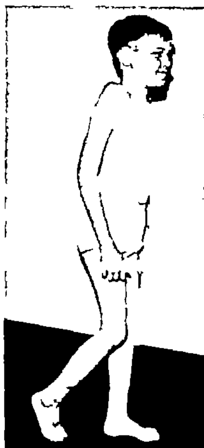


Fig. 4

learned to get up and walk with support at 8 to 14 months. Ten children learned to walk without support 9 between 10 and 20 months of age and one at 40 months, whereas 5 aged 2½ to 15 years at the latest examination, were never able to do so All the children able to walk had an abnormal waddling gait with a tendency to overstretch the knees, lumbar lordosis and a protuberant abdomen (Fig. 4 5)

According to the histories the symptoms had been progressive for a few months in 2 patients (case 17 and 22) more slowly and for several years in the rest of the patients Thus one child (case 10) who could walk without support at 3½ years was no longer able to do so at 5. One girl (case 18) has since the age of 11 developed a progressive kyphoscoliosis and became at 11½ confined to a wheel-chair a year later atrophy and fasciculations of the tongue were first observed. Another patient (case 23) had bulbar signs when first examined at 5½ whereas none were noted in the remaining 13 patients. Three patients (cases 16 (Fig 6) 18, 23), latest examined at age 15 14 and 5½ were then handicapped almost to the same extent as the oldest child belonging to type I

Weakness and wasting always involved the extensors of the hips and knees,



Fig. 4 b.

Fig. 4 A and B. Case 19 Age 11 years.

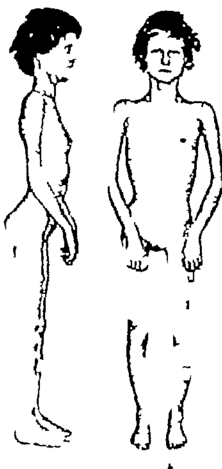


Fig. 2A and B Case 1 Age 12 years.

the pelvic muscles and the lower back muscles first and most severely Involvement of the muscles of the shoulder girdle was obvious in L patients, minimal in one (case 18) and equivocal in one (case 14) (these were examined at 3½ years) In one patient (case 1) weakness of the shoulder girdle was uncertain at 18 month but obvious years later

Muscle reflexes were lost or weak in 14 patients. The quadriceps reflexes usually disappeared first and the calf reflexes were best preserved One patient (case 14) had normal reflexes and was also the one handicapped least in the group

The patients of type II are all alive and were 3½-15 years of age at the latest examination.

No reflexes abnormal for age were demonstrable in patients of type I or II.

One patient (case 25) was classified as type III or juvenile amyotrophic lateral sclerosis. His development during infancy was normal, but he could not walk without support until he was . For several years he remained rather well although he had difficulties in ascending stairs and running From age 13-14 his condition deteriorated When first seen at age 16 he had severe genera-



Fig. 3 Case 15 Age 14 years



Fig 7 Case 13 Age 16 years.

lized weakness and wasting, most pronounced in the muscles of the pelvic and shoulder girdles (Fig. 7) He lifted *his head with good strength and had no* spinal deformities. He could walk only a few steps without support. *Muscle reflexes were brisk and Babinski's sign was present bilaterally* He had no bulbar signs. He was followed up for 4 years during which further deterioration was noted.

Extra-ocular and facial muscles were normal in all 25 patients. Sensation and mental development appeared normal in all patients old enough to be judged in these respects.

*Radiological findings* The degree of muscular atrophy was often hard to evaluate clinically in young infants because of the small muscles and firm subcutaneous fat at this age in some of the older children, because of obesity X ray of the limbs showed thin muscles with fat streaks between the muscle bundles, i.e. signs of reduced muscle mass also in those patients in whom this was hard to detect clinically (Fig 8) Those children who had a long-standing severe muscle disease and were unable to move also had thin decalcified bones.

*Laboratory studies* The spinal fluid contained 0–3 cells/mm and a protein concentration normal for age

The *activity of the serum transaminases* was measured in 20 patients and was normal in all Lactic dehydrogenase determined in 19 patients, was normal in 7 In 12 the total activity was elevated (350–870 U normal 300 U) with a re-




Fig 8. Case 9 Radiological picture of left forearm (A) and right calf (B) Note the thin decalcified bones and the thick fat layer with streaks between the atrophic muscle bundles.

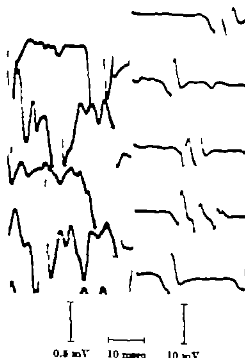



Fig 9. Case 14. Electromyogram from the right first dorsal interosseus muscle; motor unit potentials recorded during attempted maximal contraction. The left part of the figure demonstrates the decreased number and long duration of motor unit potentials. The amplification in this part of the figure too high t permit evaluation of the amplitude. The high amplitude of motor unit potentials is demonstrated in the right part of the figure where amplification is lower

lative increase of the 3 first isoenzymes, particularly isoenzyme 1 Creatine kinase was normal in 1 and increased i.e. 2-3 times the upper normal limit in 3 of 15 patients.

*Neurophysiological examination* The conduction velocity of peripheral nerves was normal in 10 patients In 6, all severely affected and 4 of them infant of type 1 1-4 borderline and 1-4 slightly abnormal values were found. In 4 patient the first electromyographic examination revealed abnormalities typical of denervation, i.e., the presence of fibrillations, which in size and number exceeded the small fibrillations occasionally found in distal muscles of young infants, and motor unit potentials of high amplitude and long mean duration for age a reduced number of motor unit potentials of high frequency was found during attempted maximal contraction (Fig 9) One patient was examined at 5 9 and 13 months. The findings at the first 2 examinations were consistent with a diagnosis of denervation, but not until the third examination were the findings pronounced enough to permit a firm diagnosis.

*Histopathology* Histologically judgeable muscle biopsy specimens were obtained in 21 patients. The piece xammed was normal in one patient (case 13) In 16

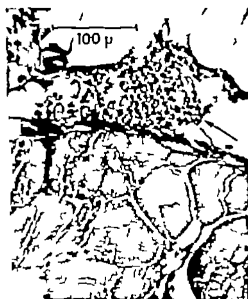



Fig 10 ( se 19 Biopsy from the anterior tibial muscle The arrow points at group of atrophic f bres Normal f bres are seen below the group of trophic f bres ( 225, Hansen-v Gieson)

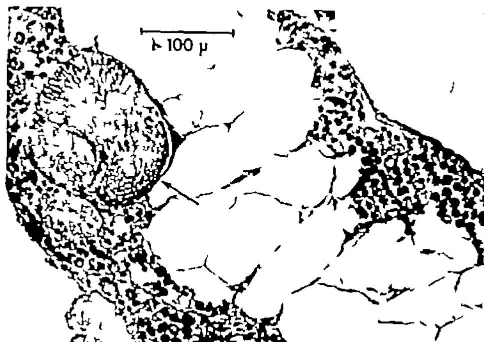



Fig 11 Case 16 Biopsy from the anterio tibial muscle. Almost all of the fibres are atrophic The arrow points t hypertrophic fibre with centrally placed nuclei nd signs of muscle fibre splitting ( 225, Hansen v Gieson).

patients atrophic fibres in large groups, characteristic of denervation, were found (Fig 10) In 4 patients this pattern was combined with findings usually interpreted as signs of primary myopathy e.g fibres with central rows of nuclei, hypertrophic fibres and splitting fibres (Fig 11) Three of these patients (cases 9 12 16) were older children and had had symptoms for at least 12 years the fourth (case 23) was a severely affected boy who had had symptoms for about 5 years.

## Discussion

Is progressive spinal muscular atrophy one disease or can different independent entities be distinguished on genetical and clinical grounds? The independence of the juvenile type has been stressed because of its later onset and more benign course [3 10 12, 13 30 33, 35 40 58 50] and different mode of inheritance [4].

The occurrence of cases running a course between the infantile and juvenile types is, however evident from larger reviews [6, 8, 28, 51] and from case reports [9 34 48 50] and has been particularly stressed by Dubowitz [16] Infantile and juvenile cases have been reported in the same family [2 10, 10 20, 36] On the basis of clinical and genetical studies of 83 cases of progressive spinal muscular atrophy Hausmanowa Petrusewicz *et al* [?0] concluded that different types overlap one another so widely that a variability in the manifestations of a single disease appears the most likely interpretation The present material does not include enough older patients to permit a firm conclusion There was, however considerable overlapping be-

tween the 3 different types and of 2 brothers one belonged to type I and the other to type II. It appears therefore likely that children, in whom symptoms of progressive spinal muscular atrophy are noticed during their first 3 years of life all have the same disease with the possible exception of those in whom dominant inheritance is evident.

The inheritance is usually described as autosomal recessive both in infantile and juvenile cases. However Becker [4] pointed out that published materials of juvenile cases seem rather to support the assumption of irregular dominant inheritance a difference from the infantile cases which he stresses as proof of separate diseases. Regular dominant inheritance has also been reported [3 53]. Most of the present cases were either sporadic or seen in patients with affected sibs i.e. the findings were compatible with recessive inheritance. However one patient (case 14) has an affected father and dominant inheritance is thus likely. It is of interest that this patient had the mildest manifestation of the disease in the whole material, thus supporting the view [4] that a particularly benign form may exist as a separate disease with dominant inheritance.

The occurrence of pyramidal tract signs in one juvenile case of progressive spinal muscular atrophy is of clinical interest because it underlines the fact that muscle reflexes may be normal hypo- or hyperactive in cases of lower motor neurone disease depending on the presence or absence of coexisting upper motor neurone involvement. In this patient the clinical course was more similar to that of juvenile spinal muscular atrophy than to that of amyotrophic lateral sclerosis in adulthood. He was the oldest patient in the material. It is perhaps possible that also other patients develop upper motor neurone symptoms with increasing age. Widespread degenerative lesions with in both grey and white matter have been demonstrated neuropathologically in cases of infantile progressive spinal muscular atrophy [38 39 4 ]

The purpose of the present classification was to define the early symptoms which may help to predict the further course in a given case. A low age at onset is believed to be prognostically unfavourable a belief corroborated to some extent by this material. However the stated age at onset may be unreliable because of the early onset and insidious course of the disease. The early and severe involvement of neck and trunk muscles preventing the children from lifting the head and learning to sit up symptoms used here to define type I seemed to carry an unfavourable prognosis. If the dominating initial symptoms were thin thighs and weak kicking symptoms used here to define type II the prognosis appeared better even when these symptoms were observed early perhaps already in the neonatal period. Although Byers & Banker [8] used the age at onset as the main basis for classification the same difference in prognosis was apparent between their patients with early generalized weakness and those with weakness localized mainly to the girdle and leg muscles.

The finding of normal transaminases in cases of spinal muscular atrophy is in agreement with earlier reports [14 20 45]. A normal activity of creatine

kinase in cases of spinal muscular atrophy *has been stressed as a sign differentiating* them from cases of primary myopathy [22 44 5_] Increased activity has, however been reported [28, 53], and the finding of a slight to moderate increase in 3 of 15 patients is thus not surprising The increase was never of the magnitude usually seen in severe cases of primary myopathy in this age group [1 15 18 22 44] An elevated total activity of lactic dehydrogenase with a relative increase of the first 3 isoenzymes, particularly isoenzyme 1 seen in 9/3 of the patients is a good differential sign [18] from the distinct increase of isoenzyme 2 regularly found in children with severe primary myopathy [18 46 57 61]

The occasional finding of a slightly decreased conduction velocity of peripheral nerves in patients severely affected with spinal muscular atrophy has a technical explanation [31] The velocity recorded is that of the thickest and fastest conducting motor fibres of the nerve as the disease attacks the largest anterior horn cells with the largest nerve fibres most severely healthy but thinner nerve fibres will be the fastest conducting ones and a lower conduction velocity will be recorded. It is important to examine electromyographically both arm and leg muscles particularly in those patients who have equivocal clinical findings in the shoulder girdle as weakness and denervation only in leg muscles may be caused by a restricted lesion in the spinal cord or the ventral roots

The occurrence in denervated muscles of histopathologic abnormalities, usually ascribed to a primary myopathy as in 1/5 of the material presented here has been described previously and may be a common finding in long-standing cases [7 28, 37]

## Summary

The results of clinical, radiologic, laboratory neurophysiologic and histopathologic studies are reported in 25 patients with progressive spinal muscular atrophy beginning within the first 3 years of life At the first examination the patients were between 4 months and 16 years of age The findings suggest that progressive spinal muscular atrophy with an early onset is one disease, though the course and prognosis may vary

On the basis of the clinical findings 3 different types could be distinguished. Type I was characterized by early onset of severe *generalized* weakness, involving the neck, trunk and proximal arm and leg muscles. The first symptoms were muscular hypotonia and inability to lift the head, elbows or knees from the bed. Of 9 children belonging to this type 4 died before age 1 and 1 at age 3 none learned to walk. Type II was characterized by onset in some patients already in the neonatal period of *localized* weakness, confined to the hip girdle and proximal leg muscles. The first symptoms were weak kicking and thin thighs Fifteen patients belonged to this type *all* were alive at the end of the observation period However the disease was progressive at least over several years also in this type and some children were as severely handicapped as the oldest surviving child belonging to type I. Of 2 brothers one belonged to type I the other to type II Type III to which one patient belonged, was characterized by the appearance of

pyramidal tract signs. The clinical picture was otherwise similar to that seen in patients of type II.

The serum transaminases were normal creatine kinase was increased in 3 of 15 examined patients, lactic dehydrogenase was normal in 7 of 19 examined patients and slightly elevated with a relative increase of the first 2 isoenzymes, mainly isoenzyme 1 in the remaining 12. The conduction velocity of peripheral motor nerves was normal in 10 patients and borderline low or slightly decreased in 6. Histopathologic examination of a muscle biopsy specimen was normal in one patient. The remaining patients showed large groups of atrophic fibres; in 4 this pattern was combined with abnormalities usually interpreted as signs of a primary muscle disease.

The special studies revealed no difference between the 3 types

## Acknowledgement

Dr Ann Vogt determined the activity in the serum of lactic dehydrogenase and performed the separation in isoenzymes; Dr Kjell Sjövall determined the activity in the serum of creatine kinase Dr K.-G Henriksson performed the histopathologic studies of the muscle biopsy specimens.

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fibrosis were absent at autopsy This may be due to the fact that a marrow specimen was then taken from a single bone only (the femur) [2]

Randall *et al* [13] have recently described a new syndrome "familial myeloproliferative disease The present case resembles this condition in many ways especially in the early onset of splenomegaly anaemia and thrombocytopenia and in the myeloid leukaemic picture at autopsy But the extremely high percentage of immature red blood cells and the signs of myelofibrosis in our patient were not described in any of Randall's cases. The possibility of a familial aspect of the disease in our patient cannot be stated.

The most likely diagnosis in the present case must then be myelofibrosis passing terminally into a myeloid leukaemic state. The relation between these two conditions, as well as between other myeloproliferative disorders, is not fully understood, but the theory proposed by Dameshek [5] seems to be useful. According to this the bone marrow cells often proliferate *en masse* or as a unit rather than as single elements. Myeloid leukaemia may then be just one type of proliferation myelofibrosis another eventually provoked by a myelostimulatory principle perhaps of hormonal, perhaps of exogenous (toxic) nature

## Summary

A case of myelofibrosis is described in a boy aged 3½ years. At some time towards the terminal stage the condition passed into one of myeloid leukaemia. Such a relationship between myelofibrosis and myeloid leukaemia has not been described before in children. The disease was characterized by spleno- and hepatomegaly anaemia pronounced thrombocytopenia and by an excessive rise of the total number of red nucleated cells in the peripheral blood.

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CASE REPORT

# A Case of Thymic Alymphoplasia with Synthesis of IgM Globulin

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In 1950 Glanzmann & Riniker [7] described a fatal disease in two infants, both of whom showed high susceptibility to infection and had Candida albicans infection which was refractory to treatment. In both cases there was marked lymphopenia verified by examination of the patients on several occasions the lymphocyte count being less than 1000 per mm³ The postmortem findings included pronounced hypoplasia of all lymphoid tissues in both cases. The authors believed that the disease represented a clinical entity and called it essentielle Lymphocytophthise In 1958 Tobler & Cottier [15] reported 2 cases of a similar disease the patients being siblings of the two infants described by Glanzmann & Riniker Both infants had pronounced lymphopenia Immunoelectrophoresis performed on one of them at the age of seven months, showed almost complete agammaglobulinaemia In 1961 Hitzig & Willi [8] reviewed the cases published in the literature up to that year and found 9 to be authentic and 22 to be probable cases of agammaglobulinaemia with co-existent lymphopenia

According to Gitlin & Craig [6] there is a difference between the cases of agammaglobulinaemia associated with lymphopenia and those of agammaglobulinaemia alone in respect to the clinical course and to the postmortem findings. In the former group the disease appears early in infancy and is fatal. There is marked and persistent lymphopenia, a rudimentary thymus and generalised lymphoid hypoplasia small lymphocytes being scantily present in the other lymphoid tissues. In the latter group the disease is intermittent and considerably less severe in character; there is only occasional and transient lymphopenia in peripheral blood and the number of lymphocytes is virtually normal in the thymus and in the other lymphoid tissues. Germinal follicles are absent and plasma cells are not demonstrable in both groups.

Patients with agammaglobulinaemia associated with lymphopenia show inability to manifest delayed hypersensitivity and do not reject homologous transplant [13]. Transplantation of thymus tissue has been performed without effect on either the lymphopenia or the clinical course [13].

The first cases were reported from Switzerland but the disease has also been observed in the U.S.A [5 6 13], England [13], France [11 14] and Sweden [4] The

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mode of inheritance is probably as an autosomal recessive character in some families and a sexlinked recessive one in others [1,].

This report describes a case of agammaglobulinaemia associated with lymphopenia in which the concentration of IgM rose to a subnormal level as verified by repeated examinations of the patient

## Case Report

The patient, a girl, born on June 6, 1965, was the first child of healthy non-consanguinous parents. Weight at birth was 2,950 g. Except for allergic disease and psoriasis in the mother family the family history was negati e. The patient thrived initially and feeding did not involve any problems. BCG vaccination was performed when the patient was a few days old. At the age of three weeks loose and frequent stools were noted and weight-gain was unsatisfactory. Concurrently the patient developed paronychia which involved several fingers, and this was resistant t treatment. At the age of eight weeks she was admitted t the Department of Paediatric Surgery at the University Hospital in Uppsala for treatment of the paronychia. A focus of osteitis was removed from one finger. Bacterial culture showed the presence of *Staph. aureus*. Treatment with antibiotics was effecti in eradicating the paronychia. From the age of two months the patient had persistent rhinorroea and pertussoid cough. On September 9 1965 she was admitted t the Department of Paediatrics at the University Hospit l in Uppsala.

*Condition on admission*

Physical examination showed poorly nourished infant who became cyanotic only when crying. Respirations were 80 per minute. The lymph nodes were not enlarged. The throat w slightly reddened and a mucopurulent discharge was present. There was nothing noteworthy bout the respiratory sounds.

*Laboratory findings* On admission the haemoglobin was 15 g per 100 ml. Thereafter it gradually fell to 10.4 g per 100 ml and then rose to 1..2 g per 100 ml. The highest white blood count was 16,900 per mm³. There was marked relative as well a bsolute lymphopenia, the count of lymphocytes ranging between 200 and 600 per mm. The number of neutrophil leukocytes was normal. Immunoelectrophoresis revealed the concentration of IgG to be low in relation to the patient age IgA and IgM were not demonstrable. Isoagglutinins could not be demonstrated. (The blood group was O Rh positive.) The Widal reaction was negative following three injections of polyvalent typhoid vaccine. The sensitisation test with dinitrofluorbenzene was negative. Bone marrow aspiration revealed 93 600 nucleated cells per mm³ active normoblastic erythropoesis, fairly active myelopoiesis, which was displaced to the left and the presence f ll mature forms. The eosinophils were slightly increased. Very few lymphocytes were found and plasma cells were not identified. X-ray examination of the chest showed slowly progressive changes in the form of desera tion and band like densities in both lungs, but there was no evidence of a tendency towards confluent infiltration. The outline of the thymus was not visible in the upper mediastinum. The skin test with tuberculin was negat ve on two occasions (September 9 and Oct ber 7). Electrocardiography did not reveal any bnormality initially. Cultures of the throat feces and urine were positi for Candida albicans on several occasions and showed abundant growth f *Pseudomonas aeruginosa*. Urinalysis disclosed abundant *E. col* on several occasions

*Clinical course*

The patient showed tachypnoea and tended to become cyanotic. These symptoms persisted until her death. Feeding and crying resulted in great distress and she had t be fed with stomach tube. The stools continued to be loose and frequent despit the various diets which were tried, and weight gain wa minimal. There was persistent

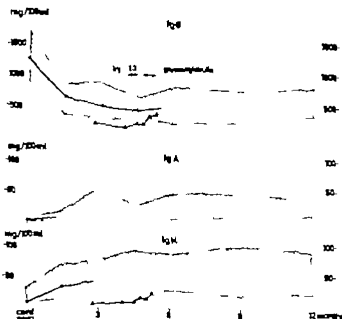


Fig. 1. The concentrations of the immune globulins in the patient compared with the results for healthy infants.

rhinorrhoea and a pertussoid cough. A co-existent severe monilial infection of the oral mucous membranes was refractory to treatment. The tonsils were remarkably small. BCG vaccination produced a strong local reaction and at the age of three months the patient developed an abscess in the groin on the side on which she had been vaccinated. Direct microscopy of the pus exuded by the abscess showed the presence of numerous acid fast rods. Culture of the pus according to the method of Löwenstein was positive but guinea pig inoculation was negative. Culture according to the method of Löwenstein and guinea pig inoculation of gastric washings was negative.

The illness was refractory to treatment. The patient was given sulphonamide preparations and various antibiotics. During the week prior to death she was given a combination of streptomycin, PAS and isoniazid. The monilial infection was treated with large doses of nystatin and locally with gentian violet without any appreciable effect. Several doses of gammaglobulin were given. During the month preceding the patient's death, signs and symptoms of an hepatic lesion developed which became increasingly severe in character. These included a rise in serum transaminases and in the directly acting serum bilirubin level, icterus, pale stools and darkcoloured urine. On November 22 the patient died showing cardiac insufficiency with oedema, hepatosplenomegaly and dyspnoea.

*Immunological studies*

Samples of serum were tested for immune globulins by immunoelectrophoresis and quantitative determinations of the different immune globulins were made by single radial diffusion in gel applying a modification [9] of the method of Mancini *et al.* [10].

In Fig. 1 the concentrations of the immune globulins are compared with the results for healthy infants [2].

*Post-mortem findings*

*Superficial lymphnodes.* A few hypoplastic lymph nodes were present, the largest being hardly the size of a peppercorn. Histologically they all showed lymphoid hypoplasia

and hyperplasia of the reticulo-endothelial elements. Clusters of large histocytic cells showing abundant cytoplasm were present, particularly in the sinusoids of the lymph nodes from the intestinal mesentery. *Thymus.* A few nodules, each being smaller than a pea with a total weight of 1.5 g, were found at the site of the thymus. Microscopy showed that this tissue lacked the ordinary structure of cortex and medulla. There was marked lymphoid hypoplasia, only a few and widely separated clusters of cells resembling lymphocytes being present. There were only a few Hassall bodies which were markedly atrophic. As in the superficial lymph nodes there was hyperplasia of the reticuloendothelial elements. *Spleen.* Microscopy showed almost complet absence of follicles and hyperplasia of the reticulo-endothelial elements. *Intestine.* Microscopy revealed the absence of lymphoid follicles and plasma cells. *Lungs.* The histological picture resembled that of Pneumocystis carinii pneumonia but special staining for the demonstration of Pneumocystis carinii failed to reveal the presence of these organisms. *Microscopy of tissue samples from the left groin.* There wa no evidence of infiltration with epithelioid cells and giant cells such as Langhans cells were not seen. Staining for tubercle bacilli revealed bundant acid-fast rods, some being phagocytised within macrophages, others lying free

## Discussion

In the case presented the diagnosis of typical lymphocytopenic agammaglobulinaemia (Swiss type) was made from the course of the disease the marked lymphocytopenia and the post-mortem findings. There probably was a relationship between our patient s strong reaction to BCG vacci nation and her primary disease As generalised infection due to BCG inocula tion was suspected, the patient was treat ed with a combination of streptomycine PAS and isoniazid However the post mortem findings and the results of cultures of different tissues according to the method of Löwenstein did not support this presumptive diagnosis. This might have been due to the treatment given.

The decrease in the concentration of IgG globulin in our patient between the age of 2½ and 3½ months was considered to suggest hypogammaglobulinaemia with scanty production of IgG globulin by the patient. The patient was therefore treated with large doses of gammaglobulin during the course of five weeks, receiving a total dose of 4.7 g This resulted in a rise of the IgG concentration from 150 mg per 100 ml to 3?0 mg per 100 ml Up to the age of four months the concentration of IgM globulin ranged between 6 mg per 100 ml and 10 mg per 100 ml but shortly before the patient died it rose to 23 mg per 100 ml. The concentration of IgM was 31 mg per 100 ml in the solution of gammaglobu lin injected. Disregarding catabolism of IgM the injection of 80 ml of this solution of gammaglobulin would be required in order to obtain a concentration of 23 mg per 100 ml of IgM Between the age of four and five months the patient was given only 28 ml of such sol tion. For this rea son the marked rise in the IgM concentra tion cannot have been due to the presence of IgM globulin in the solution of gamma globulin injected but indicates synthesis of IgM by the patient The concentrations of IgM globulin in serum from 23 healthy infants between the ges of four and six months were found to range between ?0 mg per 100 ml and 97 mg per 100 ml the mean alue being 40 mg per 100 ml [ ]. Consequently the IgM synthesis was delayed in our patient but the lower limit of normal value w s reached before the age of five months.

Repeated determinations of the concentration of IgA globulin consistently showed very low levels of this globulin, the maximum concentration being 3 mg per 100 ml. At no stage of the disease could IgD globulin be demonstrated. Specific antibodies were not demonstrable The patient lacked isoagglutinins. Antibodies against H or O antigen were not identified despite repeated inoculations of polyvalent typhoid vaccine The patient's inability to manifest delayed hypersensitivity was demonstrated by the negative tuberculin test carried out after BCG vaccination and failure of dinitrofluorbenzene to result in hypersensitivity

Recently Fireman *et al* [5] described a case of agammaglobulinaemia associated with lymphopenia with remarkably high concentration of IgM, i.e. 200 mg per 100 ml. The concentration of IgG and IgA globulin respectively was remarkably low The post mortem findings were characteristic of thymic alymphoplasia except for the presence of a few lymph nodes of normal size in the mesentery which contained an unusually large number of plasma cells. Plasma cells were also present in the spleen.

Nezelof *et al.* [11] described a case of thymic lymphoplasia in which plasma cells were present and the concentrations of all immune globulins were normal.

In our case plasma cells were not demonstrable nor did the post-mortem findings disclose the tissue in which the IgM globulin was produced.

## Summary

A female infant with agammaglobulinaemia associated with lymphopenia causing the death of the patient at the age of five months is described. The concentrations of the different immune globulins were determined on several occasions, the last determination being carried out shortly before the patient's death. Whilst synthesis of IgG and IgA globulin respectively by the patient was very low the concentration of IgM globulin began to rise from the age of four months, subnormal values being found shortly before the patient's death. At no stage of the disease was IgD globulin demonstrable

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CASE REPORT

# Intravascular Coagulation in an Infant with the Hemolytic Uremic Syndrome

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The hemolytic-uremic syndrome is characterized clinically by the association of severe intravascular hemolysis, nephropathy and, frequently a hemorrhagic diathesis.

Intravascular coagulation is suggested to play a primary role in the pathophysiology of this syndrome [25]

A new case of a patient suffering from the hemolytic-uremic syndrome is described, in which examination gave strong support for the existence of a transitory intravascular coagulation.

## Methods

Plasma for coagulation studies was prepared from 9 parts of blood and 1 part of 3.8% trisodiumcitrat dihydrat mixed immediately and centrifugated for 20 min at 2,000 g. Serum was obtained from blood clotted in glass tubes and incubated during 2 hrs at 37°C and 24 hrs at room temperature.

Platelet count was done according to Feissly [5], factor I (fibrinogen) according to Clauss–Vermijlen [2, 33], factor II (prothrombin) and factor V (proaccelerin) was determined with one-stage methods according to Koller [1?] and Stormorken [31]. Factor VIII (antihemophilic factor A) was determined according to Langdell & Margolis using deficient plasma samples as test plasma [14, 19].

Fibrin split products were determined immunologically according to Ferreira-Murat [6].

## Case History

*1st Admission*

The patient born Nov. 4th 1964, was in perfect health until June 1965, when diarrhea occurred, which was unresponsive to chlorquinaldolum (Sterosan®) and sulf drugs. The family history was non contributory.

Because of an increasing pallor the patient was admitted elsewhere on *June 12th 1965*. The laboratory result were as follows: hemoglobin 3.9 g/100 ml, erythrocytes 1.3 million/mm, platelets 14,000/mm and leucocytes 18,500/mm with a differential count of: 3 myelocytes, 7 metamyelocytes, 26 unsegmented neutrophils, 26 segmented neutrophils, 37 lymphocytes and 1 monocyt. 300 ml blood was transfused after which the patient was referred to our department on June 14th 1965.

Examination revealed a pale moderately ill infant the skin turgidity was good; there were no petechiae. The sclerae were slightly icteric. The blood pressure was 100/60 mm Hg. The liver edge was felt 2 fingers beneath the right costal margin, the spleen was just palpable. The reflexes were symmetrically increased.

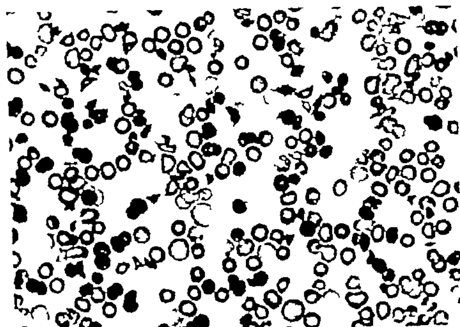


Fig. 1 Blood film showing anisocytosis, poikilocytosis and irregularly contracted red cells

The following laboratory results were obtained: hemoglobin: 9.2 g/100 ml; reticulocytes 120 ‰, normoblasts 20 per 100 leucocytes; leucocytes 12,960/mm³; platelets 23,000/mm³; blood urea 287 mg/100 ml, electrolytes N 126, K 4.3 Cl 83 4 HCO 16.7 mEq/l, serum bilirubin 1.33 mg/100 ml indirect; haptoglobin was greatly diminished. The blood film showed bizarre shapes of red cells anisocytosis, poikilocytosis and irregularly contracted red cells (Fig. 1). The bone marrow revealed a remarkably active erythroid component. The number of megakaryocytes was normal. Urinalysis revealed the presence of 2.5 g/l protein. Microscopic examination of the urinary sediment showed many red blood cells and few whit blood cells. Granular casts were present

The diagnosis of hemolytic-uremic syndrome was made. The result of the blood clotting tests which were performed are shown in Table I. There was marked decrease of circulating platelets and a moderate decrease of fibrinogen and prothrombin There was also a marked rise of antihemophilic factor A act vity an increase in antithrombin activity and presence of circulat ing fibrin split products. All these clotting abnormalities are described in intra vascular clotting and gave circumstantial evidence for the existence of disseminated clotting. Spontaneous normalisation of the results of the coagulation studies contraindicated administration of heparin.

The infant was treated conserv t vely with diet, packed red cells and correction of the electrolytes

The patient was discharged on Sept 9th 1965 th hemoglobin of 13 1 g 100 ml, plat let count of 254 000 mm nd no bnormalities in the urinary sediment

The endogenous creatinine clearance mounted to 73 8 and 73.2 ml/min 1 73 m²

*nd Admissi on*

The patient was readmitted in F br 1966 for renal function control. The blood pressure was 120/70 mm Hg The hemoglobin level

TABLE 1 *Coagulation studies in a case of hemolytic uremic syndrome in comparison to normal results*

| | 15-6-1965 | 16-6-1965 | 17-6-1965 | 24-6-1965 | Normal |
|---|---|---|---|---|---|
| Clotting time min | 4½ | — | — | — | 4–7 |
| Platelets per mm³ | 22,000 | — | 100,000 | 180,000 | 150–300,000 |
| Factor I mg/100 ml | — | 133 | 128 | 173 | 200–400 |
| *Factor II* | — | 80 | 100 | 100 | 80–120 |
| Factor V % | — | 100 | 100 | 100 | 80–120 |
| Factor VIII % | — | 200 | 200 | 100 | 80–120 |
| Trombin time sec | — | — | 40 | 26 | 19–20 |
| Fibrin split-products | — | pos. | pos | neg. | neg. |
| Blood urea N mg/100 ml | 207 | 380 | 336 | 83 | |

was 15 g/100 ml. There were 296,000 platelets/mm

The endogenous creatinine clearance amounted to 81.5–82.2–112.8 ml/min/1.73 m. An intravenous pyelogram revealed no abnormalities. The renal dilution capacity was normal. A urine concentration test revealed an osmolality f 956 mmol.

No abnormalities were f und in the uri nary sediment

A renal biopsy was not llowed.

## Discussion

The symptomatology in this patient corresponded to the hemolytic-uremic syndrome. A prodromal phase of gastroenteritis was followed by a severe anemia. The hemolytic character of the anemia was apparent from the marked reticulocytosis, the raised indirect bilirubin level, the strongly diminished free haptoglobin and the rapid fall of the Hgb level aft r bloodtransfusion, without external blood loss apart from the hematuria (within 3 days the Hgb level fell from 9.. to 6.1 g/100 ml). The abnormalities in the shape of the red cells were identical to those described by Brain *et al.* [1] in microangiopathy. In addition there was renal insufficiency and thrombocytopenia.

The low platelet count, the low level of fibrinogen and prothrombin, together with the presence of fibrin split products in our patient, are evidence of disseminated intravascular coagulation [28, 30]. Sometimes a high level of antihemophilic factor A is found in intravascular coagulation [24, 10].

In a patient suffering from the hemolytic-uremic syndrome Desmit *et al.* [4] found a fall in the number of platelets, the fibrinogen level and the prothrombin level. During heparin administration, they observed in the course of one week, a rise in the platelet count from 20,000/mm³ to 150,000/mm³ while the fibrinogen level increased from 88 to 370 mg/100 ml. Piel & Phibbs [25] also report a thrombocytopenia and hypothrombinemia with an increase in the number of platelets after heparin administration. Likewise Kunzer & Aalaam [13] Kibel & Barnard [11] noted an increase in the platelet count after heparin therapy.

In our patient however a spontaneous increase in the number of platelets was noted. The effect of heparin must therefore be interpreted with the necessary caution.

The known blood coagulation data make

the occurrence of intravascular coagulation in the hemolytic uremic syndrome acceptable In mild cases intravascular coagulation perhaps cannot be demonstrated [20]. The nature of this stimulus initiating the intravascular coagulation remains unexplained. There are several hypotheses. It is frequently suggested that this syndrome represents the human equivalent of the Sanarelli-Shwartzman reaction.

Experimentally the Sanarelli-Shwartzman reaction is induced in rabbits by intravenous injection of two properly spaced doses of bacterial endotoxin (the optimal time for the second injection is 4 hrs after the first) This results in two episodes of disseminated intravascular coagulation [ 1]. The first injection deposits thrombi in the liver lungs and spleen. The hypercoagulability with intravascular release of thrombin following the first endotoxin injection is explained by the disintegration of the platelets, by vessel wall damage with release of thromboplastic material and by the endotoxic shock as such [16]. Thromboplastin and fibrin macromolecules are removed from the circulating blood by the cells of the R.E.S and produce blockade of the R.E.S. [16, 3].

The altered blood lipids also seem to play a part in the blockade of the R.E.S [15] The second injection causes an increase in the number of thrombi in the liver lungs and spleen, and renal glomerular capillary thrombosis If the animal survives for 8 or more hours, bilateral renal cortical necrosis is found. The difference in response to the first and second injection of endotoxin could be explained by the R.E.S blockade In the presence of a "blockaded R E.S. thromboplastin and fibrin macromolecules are more slowly removed. This produces a more extensive intravascular thrombosis An inadequate fibrinolysis is necessary for the occurrence of the renal glomerular capillary thrombosis As in the hemolytic-uremic syndrome a hemolysis occurs in the experimental Shwartzman phenomenon [1].

If a generalized Shwartzman reaction could occur in man, the question remains as to the origin of a possible endotoxin in the hemolytic-uremic syndrome This syndrome in man is almost invariably preceded by a gastro-intestinal or respiratory illness, which probably represents an infection.

Sometimes, several members of the family suffer from gastroenteritis [23 27]. Several cases with this clinical picture are seen in a short period of time in a particular place [11 30]. Only in some cases pathogenic gram negative organisms are isolated. *E coli* from the urine of four patients [7 17 23] from the stools of two others [29] and from the blood of one patient [23]; *Salmonella D* from the stool of one [17]; *Proteus* bacillus from the urine of one [18]

In view of the fact that most findings are negative it is not very convincing that pathogenic bacteria play a role in this disease The second hypothesis proceeds from the fact that a [illegible]ral agent may be implicated in this disease

Gianantonio *et al* [8] have isolated a virus from the blood of [illegible] patient during the acute phase of the disease and have demonstrated in 15 patients significant antibody titres to a virus isolated from one of these patients during convalescence Virus isolation is further more reported by Rapoport [[illegible]] and Taft [32]. *Echo 29*

virus was recovered from the stools of one with a clear rise in antibody titre [9]. The majority however [20] report negative findings in spite of extensive viral examinations, so that also a viral infection remains in doubt.

Nevertheless it is known that in viral infections such as variola and varicella damage may occur to the endothelial cells of capillaries and arterioles, with thrombosis of small vessels and clotting disturbances pointing to disseminated intravascular coagulation [22]. Possibly in the hemolytic-uremic syndrome a virus primarily causes endothelial damage, marked in the kidney, with secondary signs of intravascular coagulation.

Because of the occurrence of intravascular coagulation in the patients examined so far with hemolytic-uremic syndrome heparin therapy is indicated [34] to prevent an increasing thrombosis and further renal damage. At the same time this would diminish the hemolysis probably of mechanical origin [1], thus counteracting the hypercoagulability of the blood [26].


## Summary

A 7 months old patient is described, suffering from the hemolytic-uremic syndrome.

The coagulation studies pointed to a temporary disseminated intravascular coagulation. A spontaneous correction of the clotting disorders was seen without heparin therapy. Some hypotheses are discussed, concerning the pathology of this clinical picture.

In severe cases of the hemolytic-uremic syndrome extensive coagulation studies are necessary. If the number of platelets and the amount of fibrinogen are decreased or if there are other indications for intravascular coagulation there is an indication for treatment with heparin, besides the symptomatic treatment of the uremia and anemia.



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PROCEEDINGS OF PEDIATRIC SOCIETIES

# The Finnish Pediatric Society

*Meeting Dec. 8 1966*

*Ossi Pettay* Gram-negative bacteria in hospital infections

The combat of hospital infections is gradually entering a new phase. With the help of meticulous hygiene and new antibiotics the staphylococci are continuously losing ground. Correspondingly the part played by gram-negative bacteria is increasing.

During the first 10 months of 1966 somewhat more than 6000 patient were treated at the Children Hospital in H lsinki. A total of 164 deaths occurred during this period Among these bacterial infection was the primary cause of death in 17 cases, and bacterial infection was a contributory factor in a further 30 cases.

Of the above mentioned 164 deaths 0 occurred during the first 4 hours of life *In this group infection could not be shown* to have played any part as a cause of death. If these cases, which do not apply to extra uterine lif are omitted the ratio of bacterial infection in fatal cases is 47 out of 94. In 42 cases the bacteria were gram-negative, *E col Pseudomonas aeruginosa* and *Klebsiella pneumoniae* being predominant

Of the above-mentioned 47 patients, 26 had never been outside the hospital, and another 12 had been infected after admission to the hospital. Thus a gram-negative hospital infection was either the primary cause of death or at least a contributory factor in about 40 per cent of the f tal cases.

In particular these bacteria are easily spread in a ward by various humidifiers. Milk is another possible vehicl of spread. The main difficulty n combating these gram-negative bacteria is our lack of knowledge of their epidemiology nd the lack of useful effective disinfectants.

*Erkki Klemola* Cytomegalovirus mononucleosis

An acute febrile disease on the basis of the clinical and virological findings distinguishable a a *separate entity* has been observed both in previously healthy subjects and postoperatively in patients who have received large amount of fresh or relatively fresh blood. The symptoms of this disease cytomegalovirus mononucleosis" are analysed on the basis of a series consisting of 13 previously healthy subjects and 4 patients who developed the disease *after blood transfusions and operations*. The titre of complement fixing antibodies against cytomegalovirus rose to high levels during the course of the illness. In 4 of the 5 last cases investigated cytomegalovirus wa isolated from the urine 3–4 weeks after the onset of the disease Fever was the predominant clinical feature Haematologically the disease could not be distinguished from infectious mononucleosis, but the Paul-Bunnell test was negative and neither tonsillitis nor enlarged lymph nodes were found. Symptoms of liver damage were present in all cases A year-old hild later developed hepatosplenomegaly Furthermore the studies showed that after open heart surgery subclinical cytomegalovirus infections are much more frequent than infections giving clinical symptoms.

*Elli Janssen* The etiology of atypical pneumonia i children

The role of viruses or virus-like organisms in the etiology of typical pneumonia in children was discussed. Studies performed at the Aurora Hospital on the role of deno-viruses, ornithosis virus and *Mycoplasma pneumoniae* were described. The importance of virological investigation in cases requiring hospitalization was emphasized.

*Hans Åkerblom*

# BOOK REVIEWS

*M Dawkins and B MacGregor* (editors): Gestational Age, Size and Maturity

Clinics in Developmental Medicine No 19 Spastics Society Medical Education and Information Unit in Association with William Heinemann Medical Books Ltd. 1965. 115 pages, illustrated. Price: 21s. or US$3

This book comprises 16 papers given in Oxford 1964 at symposium on the causes and associations of prematurity. Paediatricians obstetricians and pathologists, mainly from Great Britain, have given equally important contributions t the volume. The problem facing the study group was mainly t evaluat the cause and effect f disproportion of birth weight for a given gestation. During recent years many in vestigators have emphasized the fact that ll babies with low birth weight do not look and behave the same. This has raised the question of new inter national grouping f newborns. Meanwhile the terminology in the field is manif ld. The various authors have tried t define the different groups of babies in their studies against arious normal materials. The most common comparison is made within the group of babies with birth weight less than 2.5 kg where ( ) the birthweight is appropriat for gi en gest tional age "premature (b) where the birthweight is lower than ex pected for a given gestation ( small for dat " = S.F D ) In spit of the aim t clarify the terminology a few of the authors allow themselves to use poorly defined terms. When trying t evaluat the prognosis of the two groups, word like jaundice, f t etc without any definition are used. This not only shows the difficulties in the field but also makes the book somewhat uneven. Several of the articles were of particular interest to the reviewers, and are mentioned as examples of the types f work presented. Dr McDonald Montreal, presents a provocati 8-year follow-up study f large group of babies weighing 4 lb or less at birth, which re compared as regards prenatal and early postnatal haracteristics The most striking finding is that mental ret rdation in the absence of cerebral palsy was four times as common in S.F D as in premature babies. Dr Dawkins, London, underlines the high intrapartum mortality rat for the S F D group Dr Gruenw ld, Baltimore stresses the information which he has bt ined from organ weight t autopsies. In S F D the liver is small and the brain relat vely large Dr Holt Sheffield discusses known meth

ods for assessing growth against maturity He stresses the usefulness of a neurological examination to evaluate maturity at birth Most papers have a useful and up-to-date list of references. The volume has certainly fulfilled its intention to survey present knowledge and stimulat to further studies. The field is still complex and though progress has been made we have just now reached the point where we may leave the st tement of Clementh Smith from 1953 "All you have to do is look at them to see they are different babies"

*Johan Gent and Göran Sterky*

## *S Z Levine* (ed ) Advances in Pediatrics

Year book, Medical Publishers Inc. Chicago 1966.307 pp. U.S.$11.50

Volume XIV of this well known series contains six articles dealing with pediatric problems of current interest. A comprehensive review of ulcerative colitis in childhood and adolescence is given by Broberger and Lagercrantz and is to a great extent based on work done at the pediatric clinic of Karolinska Sjukhuset in Stockholm. The late Dr Harold Jacobziner presents the experiences of the New York City Poison Control Cen er and Jonxis, Groningen, reviews the present knowledge of the hemoglobinopathies. Principles and mechanisms involved in immunization procedures with live attenuated virus vaccines are discussed by Karzon and Henderson. Of great interest is the article by Kretchmer and Greenberg dealing with "some physiologic and biochemical determinants of development The last chapter by McCrory Shibuya and Worthen is devoted to the problems of hereditary renal glomerular disease in infancy and childhood. There is no real need to recommend this volume; the articles are as usual of a very high standard, comprehen sive easy to read and with extensive lists of references.

*C G Bergstrand*

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# The Parathyroid Hormone and Aminoaciduria in Vitamin—D Deficiency Rickets

by E. A. HASSANEIN[1] and H. PATEL

Secondary hyperparathyroidism has been known to occur in vitamin-D deficiency rickets and is considered to be necessary to maintain normal serum calcium in the presence of deficient gastro-intestinal absorption of calcium [1 5]. Using intravenous calcium infusions to maintain mild hypercalcaemia with the idea of suppressing parathyroid function, a marked drop in phosphate excretion was found in nine cases of vitamin-D deficiency rickets [3]. Using the same technique Scriver *et al.* [19] reported the disappearance of hyperaminoaciduria in one case of vitamin-D deficiency rickets. However it was shown by Lavender & Pullman [16] that calcium can directly affect the kidney tubules enhancing phosphorus transport an effect not mediated by the parathyroids. No direct action of calcium on the hyperaminoaciduria is known.

Our aim has been to study the effect of parathyroid hormone on the aminoaciduria of vitamin-D deficiency rickets and to investigat the possible correlation between the aminoaciduria and the biochemical findings.

## Material and Method

Ten male patients showing vitamin D deficiency rickets were chosen for the study. Their ages ranged from 10 t 4 months. Clinically all patients showed broad epiphyses, rickety rosary varying degrees of generalised hypotonia with a history of delay in standing and walking. Radiologically all of them showed a classical rachitic picture. After admission, urine was collected for 6 hours, together with a blood sample. At the end of the sixth hour parathyroid hormone (Lilly) was administered subcutaneously in a dose of 5 units per kilogram body weight to all patients. Urine was collected separately for the following 6 hours. Another blood sample was taken two hours after the injection. Serum and urinary calcium and phosphorus were estimated in seven cases. Serum and urinary calcium were determined using the oxalate-permanganat method [13], serum and urine phosphorus by the method described by King & Wootton [13]. The glomerular filtration rat was measured in seven of the rachitic infants using the endogenous creatinine clearance technique [21]. Urinary amino acids were determined in seven cases using a qualitative and semiquantitative two-dimensional paper chromatography procedure as described by Wootton [21]. In the remaining three patients quantitative estimation of the urinary amino acid in the samples collected before and after parathyroid hormone administration was done by

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ion-exchange chromatography according to St in & Moore on a Beckman 1*0-B Automatic Amino Acid Analyzer

## Results

*Serum phosphorus* There was a slight drop in phosphate level of the sera taken after parathyroid hormone administration when compared to those before the injection.

*Serum calcium*. A slight to moderate rise ranging from 0.3–0.9 mg/100 ml was obtained in the seven patients estimated

*Urine phosphate*. A marked increase was obtained ranging from 20 to 45 per cent of the phosphate excreted in the pre-injection samples.

*Urine calcium* The calcium excretion was doubled in the first patient (S.D.) In the other six patients estimated an increase was obtained in the post-injection urine samples ranging from 15–20 per cent (Tables 1 and 2)

*Endogenous creatinine clearance*. In the seven cases estimated the increase in clearance after hormone administration amounted to 10–*0 per cent more than the clearance before the injection.

*Urinary amino acids*. In all the patients the postinjection urine sample showed further increase in the highly excreted amino acids of the pre-injection sample involving glycine, alanine, histidine ly sine and glutamic acid. The amount of increase varied in the different amino acids, and in the different patients (Fig 1) With regard to the increased excretion of tyrosine proline and phenylalanine in case 10 it might be mentioned that this patient had severe rachitic manifestation but no signs of scurvy Other amino acids appeared in the post-injection urine sample in seven of the ten rachitic infants, which were absent in the pre-injection sample as tyrosine phenylalanine ornithine citrulline and aspartic acid (Tables 1 and 2 and Fig 1)

TABLE 1 *Biochemical findings before parathyroid hormone administration in seven rachitic infants*

| | Case No. | Serum P | Serum Ca | Urinary P | Urinary Ca | Amino acid uria | GFR[a] |
|---|---|---|---|---|---|---|---|
| 1 | S.D | 2.8 | 9.5 | 150 | 8.5 | + | – |
| 2 | B C. | 2.5 | 10.0 | 178 | 6.5 | + | – |
| 3 | K. A. | 2.9 | 9.3 | 208 | 7 0 | + | – |
| 4 | Y S. | 2.8 | 10.1 | 237 | 9 0 | + | 93 |
| 5 | A. H. | 3.0 | 10.2 | 195 | 4.0 | + | 101 |
| 6 | S. M. | 2.9 | 9.5 | 222 | 7.5 | + | [illegible] |
| 7 | I L. | 3.1 | 9 9 | 190 | 7 0 | + | 95 |

All figures are expressed as mg per 100 ml.
[a] Glomerular filtration rate ml/minute measured by endogenous creatinine clearance.
\+ Aminoaciduria involving the amino acids glycine alanine histidine, lysine and glutamic acid.

TABLE 2 *Biochemical findings after parathyroid hormone administration in the same infants*

| | Case No. | Serum P | Serum Ca | Urinary P | Urinary Ca | Amino acid uria | GFR |
|---|---|---|---|---|---|---|---|
| 1 | S D | 2.65 | 10.4 | 202 | 17.5 | + + | – |
| 2 | B C | 2.40 | 10.5 | 210 | 8 5 | + + + | – |
| 3 | K. A. | 2.70 | 10.0 | 297 | 10 0 | + + + | – |
| 4 | Y S | 2.65 | 10 4 | 312 | 12.5 | + + | [illegible] |
| 5 | A. H. | 2.70 | 11 1 | 522 | 6.0 | + + + | 119 |
| 6 | S M. | 2.80 | 9 7 | 312 | 7 5 | + + | [illegible] |
| 7 | I. L. | 2 95 | 10.5 | 260 | 8.5 | + + | 109 |

\+ + Aminoaciduria with increased excretion of the excessively excreted amino acids in the pre-injection urine sample

\+ + + Aminoaciduria involving the highly excreted amino acids in the pre-injection urine sample and others as tyrosine, phenylalanine proline citrulline and aspartic acid.



Fig. 1. A diagrammatic representation is made of the effect of parathyroid hormone injection (5 units/kg body weight) on the amino acids excreted in the urine of 3 cases of vitamin-D deficiency rickets. The values are given in μ m/kg body weight over the collection period i.e. 6 hours. The glomerular filtration rate (GFR) in ml/minute in each case, is given on the right side of the figure. —— GFR before injecting the hormone; —— GFR after injecting the hormone; ◻ the excretion level of the amino acids analysed before hormone injection; ▪ the excretion level after the hormone injection.

## Discussion

In vitamin-D deficiency rickets the aminoaciduria is of the renal type with little or no rise in the plasma amino acid level [10 11]. On treatment with adequate doses of vitamin-D it takes at least three weeks before the aminoaciduria reverts to normal pattern. The degree of aminoaciduria was found to vary according to the amount of protein intake [12].

The triad of urinary findings in vitamin-D deficiency rickets consisting of hypocalciuria, phosphaturia and aminoaciduria was considered to be due to lack of vitamin D in sufficient quantities [8]. On the other hand parathyroid hormone when administered in small doses can produce hypocalciuria and phosphaturia [7]. Generalised aminoaciduria has been reported in hyperparathyroidism [18]. Indirect procedures to suppress parathyroid hormone liberation by the use of intravenous calcium infusions and analysing the resultant renal findings are open to the question of any possible direct effect of calcium ions on the kidney tubules.

Our results regarding the effect produced by the parathyroid hormone on phosphat excretion are in accordance with those found by Ellsworth & Howard [4] Milne [16] and Thompson [20]. The slight drop in serum phosphorus with a slight or moderate rise in serum calcium found in our cases is consistent with the finding of Jonxis [12] in a group of rachitic infant which he compared with the response in normal infants.

The increase in GFR of 10-%0% in

our cases is consistent with the findings of Bartter [2] and Pitts [17] though other workers [4] have found no increase in filtration rate after small doses of parathyroid hormone

We did not find any relationship between the degree of aminoaciduria and serum calcium level. Seven infants showed further excretion of amino acids in the presence of moderate or slight rise in serum calcium. Neither did we find any correlation of the serum phosphate level and the severity of aminoaciduria. Scriver *et al* [19] gave a phosphorus infusion to a child with marked hyperaminoaciduria and hypophosphataemia and noted that net tubular absorption of amino acids decreased further On the other hand cases of familial hypophosphataemic vitamin D resistant rickets frequently do not show hyperaminoaciduria [6]. The relation of hyperphosphaturia to hyperaminoaciduria is unsettled. Excessive phosphaturia unassociated with aminoaciduria is known to occur in familial hypophosphataemic vitamin-D resistant rickets [6].

In our study the parathyroid hormone could increase amino acid excretion either through increasing the glomerular filtration rate in the presence of a proximal tubular dysfunction known to exist in vitamin-D deficiency [8], or by directly blocking the reabsorption of amino acids from the tubules The response to injected parathyroid hormone by an increase in GFR together with a generalised type of hyperaminoaciduria in the majority of our patients favour the first possibility There was no evidence for a direct tubular effect.

Thus the aminoaciduria in vitamin-D deficiency rickets can be regarded as primarily due to proximal tubular dysfunction secondary to the vitamin D deficiency with a possible modifying effect through excess parathyroid hormone producing increase in the degree of glomerular filtration.

## Summary

Parathyroid hormone was administered to ten cases of vitamin D-deficiency rickets. An increase in the pre-existing aminoaciduria occurred, which could not be related to changes in the serum and urinary calcium and phosphorus. The glomerular filtration rate estimated in seven of the ten cases showed an increase ranging from 10–20 %. It is proposed that the increase in hyperaminoaciduria in our cases was effected through increased glomerular filtration in the presence of proximal tubular amino acid transport defect produced by vitamin D-deficiency

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# Synthesis of $\beta_{1E}$ and $\beta_{1C}$ Components of Complement in Human Foetuses

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Work on the chemistry of human complement has led to the identification of at least two components associated with $\beta_1$-globulins. The serum protein associated with the fourth component of complement ($C'_4$) has recently been identified as $\beta_{1E}$-globulin by Müller Eberhard & Biro [5]. One of the third component of complement ($C'_{3a}$) has been shown to be associated with $\beta_{1C}$-globulin by Müller Eberhard *et al.* [6]; evidence was obtained that $\beta_{1C}$-globulin reacts with red cells in the $EAC'_{1\ \ 4}$ state and that during this process, as well as after storage it is converted to $\beta_{1A}$-globulin [2 4].

The present investigation is concerned with the synthesis of these two components in human foetuses.

## Material and Methods

The antiserum to $\beta_{1E}$ and $\beta_{1C}$-globulins was obtained as follows: rabbit red cells were first sensitized with EDTA treated horse serum at 37°C for 1 hour; the cells were then washed three times with buffered saline and incubated at 37°C for 30 minutes with human fresh serum which had been previously absorbed with rabbit red cells and treated with zymosan (1 ml serum/3 mg zymosan type A) at 37°C

Several injections of red cells treated in this way were given intravenously t a rabbit.

The rabbit serum (M64) was heated at 56°C for 30 minutes and was then, if necessary absorbed with human red cells, previously washed.

The antiserum was tested with immunoelectrophoresis (Ionagar 0 8/100 ml buffer I 0 05, pH 8 6) against fresh human serum; its reaction was compared by immunoelectrophoresis and by double diffusion in agar gel (Ouchterlony plat method) with that obtained using an antiserum to $\beta_{1E}$ and $\beta$ previously described [1].

The specificity f the antiserum was also studied by testing it against red cells coated with antibody and variou component of human complement To obtain cells presumed t be in the st te EAC' the method described by Harboe *et al* [ ] was used. briefly (a) human red cells Le(a + b ) were incubated at 37°C for 90 minutes with EDTA treated serum containing anti-$Le^a$; the cells (EA) were then washed with buffered saline and (b) ncubated at 37°C for 5 minutes with human serum previously treated w th hydrazine t inactivat C' The cells (EAC ) were washed and (c) ncubated for 15 minutes t 37° with human serum heated t 56° for 30 minutes in order to supply only C' ($EAC'_{1,4}$) The red cells were washed and tested against serial doubling solution of the antiserum (M64), one drop f the suspension of red cells and one

TABLE 1 *Agglutination of red cells in intermediate state of reaction with human complement using adult and foetal sera.*

The intermediate complexes were obtained as described in "Material and Methods Adult serum (1) and the serum from foetus 19 weeks old (2) were used as source of complement

| Intermediate complex | Dilution of anti $\beta_{1C}-\beta$ serum (expressed as reciprocals) 2 | 8 | 32 | 128 | 51 | 1,000 |
|---|---|---|---|---|---|---|
| EA | – | – | – | – | – | – |
| $EAC'_{1,4,2}$ (1) | + + + | + + + | + + | + | – | – |
| $EAC'_{1,4,2,3}$ (1) | + + + | + + | + + + | + + | + | – |
| $EAC'_{1,4,2,3}$ (2) | + + | + + | + | – | – | – |

drop of the antiserum were mixed on an opal tile and the degree of agglutination scored after 10 minutes.

The preparation of the cell complex $EAC'_{1,4,2,3}$ for testing the anti $\beta$ globulin (and anti-$\beta_{1E}$) was obtained by incubating $Le(a+b-)$ red cells with EDTA-treated anti $Le^a$ serum (as for ) the cells were washed and incubated at 37° for 30 minutes with fresh serum diluted 1 [7].

## Results

Only two lines of precipitation were observed when the antiserum was tested against fresh adult serum (Fig 1) the antiserum was assumed to be specific for $\beta_{1E}$ and $\beta_{1C}$ globulins as judged by the typical pattern of precipitation and by the results of comparing its reaction with that obtained using an antiserum against $\beta_{1E}$ and $\beta$ globulins previously described [1].

The antiserum was found to react against red cells presumed to be in EAC' state (titre expressed end point" 1 t 128) and with cells in $EAC'_{1,4}$ state (titre 1 to 51 ) (Table 1)

The presence of $\beta_{1E}$ and $\beta_1$ in human foetuses was studied by testing 14 foetal sera with immunoelectrophoresis; the age of the foetuses ranged from 9 to 24 weeks The result of testing the foetal samples is summarised in Table 2 $\beta_1$ and $\beta_{1E}$ globulins could be detected in a foetus 9 weeks of age the presence of the third and fourth components of complement in this serum was confirmed by absorbing the antiserum (M04) with the foetal serum (10 volumes of antiserum to 1 volume of foetal serum) and re-testing the mixtures against fresh adult serum, it




FIG 1 Immunoelectrophoretic pattern of an adult serum ( ) and three foetal sera from 9 (b), 19 ( ) and 22 (d) weeks old foetuses, tested against horse anti human serum (H) and rabbit anti $\beta_{1E}$ anti $\beta_{1C}$-globulins serum (M)

TABLE 2 *Results of testing the serum from 14 foetuses against anti-$\beta_{1E}$ and anti-$\beta_{1C}$ antibodies using immunoelectrophoresis*

| Foetus No. | | Foetal age (weeks) | Crown-rump length (mm) | Cause of abortion | Complement components in serum* $\beta_{1E}$ | $\beta_{1C}$ |
|---|---|---|---|---|---|---|
| 1 | F 123 | 9 | 53 | T | (+) | (+) |
| 2 | F 207 | 10 | 54 | T | – | – |
| 3 | F 256 | 11 | 67 | T | – | – |
| 4 | F 244 | 1 | 79 | S | – | – |
| 5 | F 181 | 13 | 89 | T | (+) | (+) |
| 6 | F 229 | 14 | 107 | S | – | (+) |
| 7 | F 225 | 14 | 108 | S | – | – |
| 8 | F 246 | 14 | 116 | S | (+) | (+) |
| 9 | F 240 | 15 | | T | + | + |
| 10 | F 202 | 17 | 142 | U | + + | + + |
| 11 | F 215 | 19 | 173 | S | + + | + |
| 12 | F 182 | 20 | 162 | S | + + | + + |
| 13 | F 175 | 22 | 180 | S | + + | + + |
| 14 | F 110 | 24 | | S | + + | + + |
| Adult serum | | | | | + + + | + + + |

T = therapeutical abortion; S spontaneous abortion; U = unknown.
* The intensity of the reaction is graded from – = negative; (+) = faint; + = visible + + = clear; + + + = very clear

was observed that anti $\beta_{1C}$ and anti $\beta_{1E}$ were partially inhibited. However these two components of complement were absent or were present only in traces in seven other sera from foetuses less than 14 weeks of age

$\beta_{1C}$ and $\beta_{1E}$ globulins were clearly detected in all sera from foetuses more than 15 weeks old tested by immunoelectrophoresis.

To examine the *question of the time* of synthesis of $\beta_{1C}$ and $\beta_{1E}$-globulins a serological technique was also used as follows Le(a+b–) red cells were sensitized with EDTA-treated anti Le$^a$ serum (EA) and then incubated at 37°C for 90 minutes with the sample of serum (F215) collected from a foetus 19 weeks old (EAC'$_{1,4,2,3}$) After being washed the cells were tested against the antiserum As shown in Table I the cells were found to be agglutinated by the antiserum (titre as "end point" 1 to 32)

## Discussion

Since fresh human cord serum is lytic in presence of appropriate antibodies, it is reasonable to assume that all components of complement are present at birth

It is likely that the "faint" line of precipitation termed $\beta_{1E}$ and detected by immunoelectrophoresis in human cord serum by Hitzig [3] corresponds to $\beta_{1C}$-globulin

The present results produce evidence that $\beta_{1C}$ and $\beta_{1E}$ could be detected by agar gel precipitation in some foetuses 14 weeks of age.

The presence of the third component of complement ($\beta_1$ globulin) in older foetuses was confirmed by using a serological technique since the reaction of $\beta_{1C}$ with red cells coated with antibody imply a chain reaction which involves $C'_1$, $C'_4$ and $C'_2$, it is reasonable to assume that also these components of complement were present in the foetus 19 weeks old studied.

The question arises as to whether $\beta_{1C}$ and $\beta_{1E}$-globulins present in the foetal serum are derived from the maternal circulation or are synthetized by the foetus. Since γ G globulin appears to be virtually the only serum protein which crosses the human placenta (for references see Schultze & Heremans [8]) it may be postulated that these two components of complement are produced by the foetus. In the course of an investigation on the sites of synthesis in vitro of complement components, the liver of four foetuses between 20 and 25 weeks of age was found to be very active in producing $\beta_{1C}$ and $\beta_{1E}$-globulins as well as other serum proteins [10].

It is of interest to compare the present results with those obtained by Tachibana & Rosenberg [9] in mice. The whole complement system was found to be present in mice at birth, $C'_3$ was detected in foetal mice 18 days old and another component Hc' was shown to be synthesized between the 10th and the 17th day of gestation.

### Summary

Two components of human complement $\beta_{1C}$ and $\beta_{1E}$-globulins, were shown to be present in foetuses more than 14 weeks of age. These components were studied using a specific antiserum produced in rabbit with immunoelectrophoresis and serological techniques.

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# Colonization of the Neonate with Staphylococci and Gram Negative Bacilli

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Many papers have been published on the incidence and effects of staphylococci in the newborn infant [1 3 13]. Since the introduction of routine swabbing of infants with antistaphylococcal lotions the occurrence of clinical staphylococcal infections has decreased and there is evidence that the relationship between the two is one of cause and effect [8, 11 12]. The mechanism for the reduction in infection is usually assumed to be the elimination or marked diminution in the numbers of staphylococci [11]

Before the rupture of membranes, the foetus is normally bacteriologically sterile but rapidly becomes colonized with microorganisms during and after birth. If staphylococci are removed from the infant's skin by swabbing with anti-staphylococcal lotions, colonization must occur with organisms resistant to these agents. Many gram negative bacilli are partly or completely resistant [6] and in the absence of normal flora it is possible that these gram negative organisms might multiply unchecked. This could be clinically important as these organisms can cause overwhelming infections in neonates.

To solve the problem of the degree of colonization with gram negative bacilli, we must find whether the usual anti staphylococcal lotions do eliminate staphylococci. We must also find whether staphylococci and gram negative bacilli are competing organisms at a site

## Definitions and Rationale of Study

To avoid etymological and logical difficulties, precise definitions of the terms used are vital. The definitions used here are similar to those used by Shinefield [10] but have been applied more broadly

*Organism*. This term is used as a collective noun to refer to the class and not the individual bacterium.

*Colonization*. The presence of an organism at a [illegible] site without clinical evidence of inflammatory response. In the present study the criterion for colonization was the isolation of one or more colonies of the organism on an artificial medium which had been inoculated with a swab from the site

*Interference*. The situation where the prior presence of one organism tends to prevent later colonization with another

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organism In the present study the criterion for interference was the finding of a significantly lower rat of colonization with a subsequent organism in the presence of a prior organism than wa found when the prior organism was absent. The $\chi^2$ test has been used to analyse the data for the presence of interference The organisms to be considered are chosen and designated the first, or interfering organism and the second, or affected, organism. The organisms considered depend on the circumstances, but any organism could be considered in either role. The cases in which the first organism is present are divided into those in which the second organism appears later and those in which it does not appear The cases in which the first organism is not present are similarly subdivided. From this data a table can be constructed as follows:

| | 2nd organism appears later | 2nd organism does not appear later |
|---|---|---|
| 1st organism present | $\alpha$ | $\beta$ |
| 1st organism absent | $\gamma$ | $\delta$ |

The $\chi^2$ test may be applied to this table and will show whether the values of $\alpha$, $\beta$ $\gamma$ $\delta$ differ significantly from those expected by chance. If they do so differ this would be evidence that the presence of the first organism and the later colonization with the second organism were not independent events. Such evidence would not prove that the presence of the first organism directly affected colonization with the second organism, as both may be affected by an outside f ctor But it would show that connection exists and would suggest that further in estigation would be warranted.

## Materal and Methods

There are about 4000 deliveries annually in the public wards of the Brisbane Women s Hospital. All infant had hexachlorophene 3% applied to their skin within 24 hours of birth and again on the third and fifth days of life Most infants were discharged on the fifth day

In the study bacterial swabs were taken from the anterior nares, the umbilicus and from the rectum on the first third and fifth days of lif in 60 randomly selected full-term infants in the public wards. None of these infants showed evidence of clinical infection. There was no fixed time interval between the application of hexachlorophene and the taking of the swabs. The nasal and umbilical specimens were taken on cotton tipped swabsticks which had been dipped in nutrient broth. The rectal specimens were taken with glass tubes which had been lubricated with paraffin, and these samples were therefore from both the perianal skin and the rectum. The swabs were inoculated within an hour on the McConkey and blood agar plates which were incubated. Coliform and proteus organisms were identified on the plates by their characteristic colonies. Staphylococci were similarly identified on the blood agar plates. Colonies of staphylococci were pi ked off and tested for their coagulase reaction by the tube method.

Statistical analysis was done by the $\chi^2$ test, with modification suggested by Cochran [ ]

## Results

The results of the cultures of the swabs which were taken from the nose umbilicus and rectum on the first third and fifth days of life are shown in Table 1 The figures show the percentage of the children who grow each organism from each site on the different days In spite of the early application of anti-staphylococcal lotions many of the infants were colonized with staphylococci within 4 hours of birth. In the nose the majority of the staphylococci were coagulase negative but over half the infants carried coagulase positive staphylococci at their umbilicus Of equal interest is the rapid colonization of the rectum with gram negative or

TABLE 1 *Percentage of positive cultures obtained by swabbing various sites on the first third and fifth days of life.*

| Site | Organism | Percentage positive cultures | | |
|---|---|---|---|---|
| | | Day 1 | Day 3 | Day 3 |
| Nose | Staph. coag. neg. | 60 | 70 | 64 |
| | Staph. coag. pos. | 2 | 22 | 32 |
| | E. Coli | | 6 | 4 |
| | Proteus | 6 | 2 | 0 |
| Umbilicus | Staph. coag. neg. | 40 | 28 | 22 |
| | Staph. coag. pos. | 26 | 68 | 50 |
| | E. Coli | 32 | 40 | 40 |
| | Proteus | 8 | 14 | 22 |
| Rectum | Staph. coag. neg. | 32 | 6 | 8 |
| | Staph. coag. pos. | 2 | 0 | 0 |
| | E. Coli | 88 | 88 | 78 |
| | Proteus | 22 | 34 | 60 |

ganisms. We are at present investigating the source of those organisms.

The overall pattern of colonization is mainly gram positive in the nose gram negative in the rectum with an intermediate flora at the umbilicus. This pattern suggests a reciprocal relationship between the gram positive and gram nega-

TABLE 2. *Colonization at umbilicus*

1st organism coagulase negative staphylococci.
2nd organism gram negative rods.

| | 2nd organism present in later culture | 2nd organism not present in later culture |
|---|---|---|
| 1st organism present | 8 | 14 |
| 1st organism absent | 15 | 23 |

$\chi^2$ 0.01 with 1 degree of freedom $0.9 > p$ 0.8
Interpretation: No evidence of interference

TABLE 3 *Colonization at umbilicus*

1st organism: coagulase negative staphylococci.
2nd organism gram positive rods.

| | 2nd organism present in later culture | 2nd organism not present in later culture |
|---|---|---|
| 1st organism present | 10 | 15 |
| 1st organism absent | 12 | 21 |

$\chi^2 = 0.01$ with 1 degree of freedom $p > 0.9$
Interpretation: No evidence of interference.

tive organisms, and one factor in this could be bacterial interference. We have therefore analysed the data to see whether there is any evidence for interference. As this is a small study concerned with overall ecology rather than detailed types, we have not dealt with the gram negative organisms separately but have combined them into a composite heading of "gram negative rods". Consequently in the tables that follow we shall be concerned with the presence or absence of gram negative rods.

The method used to detect the presence of interference has been described. In each of the following tables, from Tables 2 to 7 the first, or interfering organism and the second or affected, organism are named. This is followed by the body of the table giving the observed results and the calculated value of $\chi^2$. The interpretation of the result is given with each table.

It can be seen from these tables that the presence of staphylococci in the umbilicus and nose does not prevent later colonization with gram negative organisms. It will be seen that although there were only 60 infants in this study some of

TABLE 4. *Colonization at anterior nares*

1st organism: coagulase negative staphylococci.
2nd organism: gram negative rods.

| | 2nd organism present in later culture | 2nd organism not present in later culture |
|---|---|---|
| 1st organism present | 4 | 56 |
| 1st organism absent | 0 | 31 |

$\chi^2 = 0{\cdot}64$ with 1 degree of freedom $0.5 > p > 0.3$.
Interpretation: No evidence of interference.

the results reach higher values, for instance in Table 4. This has arisen from repeated observations in the same infant. For example, if an infant showed the following pattern of colonization of his nose:

Day 1—Coag.neg.staph.—no gram neg.orgs
Day 2—Coag.neg.staph.—no gram neg.orgs
Day 3—Coag.neg.staph.—no gram neg.orgs

this would be counted as *two* instances (on day 2 and day 3) of the prior presence of coagulase negative staphylococci not followed by colonization with gram negative organisms.

TABLE 5. *Colonization at anterior nares*

1st organism: coagulase positive staphylococci.
2nd organism: gram negative rods.

| | 2nd organism present in next culture | 2nd organism not present in next culture |
|---|---|---|
| 1st organism present | 0 | 11 |
| 1st organism not present | 4 | 76 |

$\chi^2 = 0{\cdot}60$ with 1 degree of freedom $0.5 > p > 0.3$.
Interpretation: No evidence of interference.

TABLE 6. *Colonization at umbilicus.*

1st organism: gram negative rods.
2nd organism: coagulase negative staphylococci.

| | 2nd organism present in next culture | 2nd organism not present in next culture |
|---|---|---|
| 1st organism present | 10 | 17 |
| 1st organism absent | 25 | 19 |

$\chi^2 = 1.7$ with 1 degree of freedom $0.2 > p > 0.1$.
Interpretation: No evidence of interference.

In the tables so far given the staphylococcus has been considered to be the interfering organism. We have also considered the reverse situation, where gram negative organisms might interfer with later colonization by staphylococci. Only at the umbilicus are there sufficient numbers for analysis.

## Discussion

The practice in clinical medicine has been to eliminate pathogenic organisms by the direct action of antibacterial substances. The effect on bacterial ecology

TABLE 7. *Colonization at umbilicus*

1st organism: gram negative rods.
2nd organism: coagulase positive staphylococci.

| | 2nd organism present in next culture | 2nd organism not present in next culture |
|---|---|---|
| 1st organism present | 4 | 27 |
| 1st organism absent | 7 | 28 |

$\chi^2 = 0.3$ with 1 degree of freedom $0.7 > p > 0.5$.
Interpretation: No evidence of interference.

of the individual and the community has been little studied. It seems that methods which are suitable for the treatment of disease may not be ideal for healthy neonates.

In spite of the use of hexachlorophene in our hospital, we found that colonization of infants with staphylococci was frequent. Others have reported similar results [5] although Gluck et al. [4] showed a marked reduction in the colonization rate. The reduction in the amount of staphylococcal infection is apparently not due to the elimination of the causative organism. The direct effect of hexachlorophene on gram negative organisms is much less marked than that on staphylococci and other gram positive species [6]. This situation resembles that predicted by Miles [7] who suggested that the removal of gram positive organisms would allow more infection with gram negative bacilli, and this change has occurred in adults [9]. By analogy one might expect the use of a selective antibacterial substance such as hexachlorophene in neonates to produce excessive colonization with gram negative types. For this analogy to be valid, two further assumptions must be made. The first is that antibacterial lotions routinely applied to neonates do eliminate or significantly reduce the staphylococcal population. The second is that removal of the staphylococci predisposes to colonization with gram negative organisms. If these two assumptions were valid, we could find ourselves in the situation predicted by Shinefield et al. [10], that an abnormal flora of highly resistant staphylococci and gram negative organisms would result on the skins of infants. However our study shows that the staphylococci are not eliminated, and we could find no evidence that the presence of staphylococci inhibits colonization with gram negative species. The converse follows: that the absence of staphylococci does not predispose to gram negative colonization. Neither could we find evidence suggesting that the presence of coliforms interfered with later colonization by staphylococci.

These findings suggest that the two types of organism colonize independently but whether their long term carriage is affected by the presence of the other has not been answered here.

Our results do not finally exclude the possibility of interference. They do suggest that if interference between staphylococci and gram negative species exists in the neonate the reaction is weak and is unlikely to be clinically significant.


## Summary

The bacterial colonization of 50 healthy neonates has been studied. Hexachlorophene had been applied to all but the rate of colonization with staphylococci remained high. There was no evidence that prior colonization with staphylococci affected later colonization by coliform organisms. There does not appear to be any danger of massive colonization with gram negative organisms following the use of anti-staphylococcal lotions in neonates.

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# Whole Blood Glucose Determination in Newborn Infants Comparison and Evaluation of Five Different Methods

by JOHAN EK and LUDVIG N W DAAE

The different methods of determination of the whole blood glucose concentration are to a certain extent influenced by substances other than glucose. This interference is of minor importance in children and adults with a normal or increased blood glucose concentration. At low blood glucose concentrations, however the interfering substances represent a major source of error The whole blood glucose concentration in newborn infants is approximately half of the concentration found in children and adults, and in hypoglycemia in the newborn infant the concentration can be extremely low In the newborn period the concentration of interfering substances in blood is higher than in adults (glutathione, uric acid, bilirubin etc.). There is also the possibility of galactose being present in the blood in newborn infants, eventually yielding a positive error in some of the methods. Therefore, determination of whole blood glucose in newborn infants is more liable to erroneous results than in children and adults. The findings of Hjelm & Sjölin [4] and others [12] support this view These authors found a considerable difference between the results obtained with the method of Hagedorn-Jensen, the glucose-oxidase method with precipitation of the proteins by means of $Zn(OH)_2$ and by means of perchloric acid. We have not found any report where the ortho-toluidine method and the glucose-oxidase method with precipitation of the proteins by means of buffered perchloric acid has been evaluated and compared with other techniques in newborn infants.

As hypoglycemia in newborn infants represents a very serious condition, it is important to know the reliability of the different methods for determination of blood glucose The purposes of the present investigation are as follows:

1. To establish the mean values and the normal ranges of whole blood glucose concentrations in fasting normal newborn infants during the first "4–7" hours of life with some of the most commonly applied methods.
2. To evaluate whether the same methodological variations occur in infants with low birth weight as in normal newborn infants.
3. To establish the reproducibility of the laboratory methods.
4. To evaluate which method should be recommended from a pediatric point of view

## Material and Methods

The material consists of 50 normal full term infants born after a normal pregnancy and 11 infants with birth weight below 2500 g. All infants were born at Oslo City Hospital in the period September 1 to November 30, 1966. The fullterm infants were fed breastmilk and/or cowsmilk according to their weight. The infants of low birth weight got breast milk only. The feeding started from 1 to 16 hours after birth. The blood glucose concentration was estimated once when the infants were between 4 and 72 hours of age (mean age 46 hours), 8–9 hours after last feeding in the normal infants and after 3½–28 hours fast in the infants of low birth weight. Capillary blood samples were taken by the authors by heel puncture between 5 and 6.30 a.m. The samples were immediately added to the precipitating agents. Then the samples were kept in a refrigerator. The determinations of blood glucose were carried out within two hours after the collection of the blood samples.

The following five methods were employed:

1. The method of Hagedorn-Jensen [2].
2. The ortho-toluidine method as first described by Hultman [6] with modifications given by Hyvarinen & Nikkila [7].
3. The glucose-oxidase method with precipitation of the proteins by means of 0.33 *M* perchloric acid buffered with glycine as recommended by AB Kabi, Stockholm, Sweden.
4. The glucose-oxidase method with precipitation of the proteins by means of Zn(OH) according to Hjelm & de Verdier [5]. The glucose-oxidase–peroxidase reagent used in method 3 and 4 was provided by AB Kabi.
5. Dextrostix[1] Ames Company. This strip method represents a special modification of the glucose-oxidase principle. It was employed as described by the manufacturer.

Galactose was estimated by means of the galactose-oxidase method as worked out commercially by AB Kabi.

In the method of Hagedorn-Jensen the results were read on the table worked out by these authors. In the glucose-oxidase methods and in the ortho-toluidine method the calculation of the results were based upon standard solutions of glucose analyzed together with the samples.

The procedures were controlled by analyzing a test serum (Seronorm[2] Nyegaard & Co. A/S, Norway) together with the samples.

## Results

The results found in normal newborn infants with the four laboratory methods are shown in Table 1. The mean values and normal ranges of whole blood glucose concentrations in the newborn varies considerably according to the method employed. The distribution of the values obtained with the individual method follows the normal distribution curve.

Galactose was found in amounts less than 3 mg/100 ml when present in normal newborn infants. Thus the results given in Table 1 are not influenced to a noticeable degree by this sugar.

Table 2 demonstrates our findings in infants of low birth weight. The same pattern of variations is found as in the normal newborn infants. Also in this group galactose was found to be present in negligeable amounts. In two infants galactose was present (no. 9 and no. 10; 5 and 3 mg/100 ml respectively).

The mean glucose concentration in the 23 estimations in test serum was 100 mg/100 ml with the method of Hagedorn-Jensen, 95 mg/100 ml with the three other methods.

"Recommended Value" by the manufacturer was 98 mg/100 ml estimated with the glucose-oxidase method with precipitation of the proteins by means of $Zn(OH)_2$.

*Table 1 Whole blood glucose concentration (mg/100 ml) determined with four different methods in normal, fasting newborn infants 24–72 hours of age mean age 46 hours*

| Method | Obs. individuals | Mean | Standard deviation | $M \pm 2$ S.D. |
|---|---|---|---|---|
| Hagedorn-Jensen | 50 | 53.9 | 10.9 | 34–78 |
| Ortho-toluidine | 49 | 46.6 | 9.6 | 27–66 |
| Glucose-oxidase | | | | |
| HClO | 48 | 34.3 | 9.7 | 16–54 |
| $Zn(OH)_2$ | 33 | 50.3 | 6.9 | 37–64 |

*Table 2. Whole blood glucose concentration (mg/100 ml) determined with five different methods in fasting newborn infants of low birth weight 24–72 hours of age.*

| No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Body weight (g) | 1290 | 1880 | 1800 | 1885 | 2000 | 2020 | 2050 | 2320 | 2390 | 2450 | 2490 | 2023 |
| Age, hours | 40 | 60 | 45 | 7* | 46 | 46 | 28 | 70 | 24 | 28 | 46 | 45 |
| Fast hours | 4 | 4 | 3½ | 4 | 4 | 4 | 28 | 6 | 7 | 28 | 6 | — |
| Hagedorn-Jensen | 80 | 69 | 51 | 65 | 51 | 65 | — | 59 | 68 | 45 | 69 | 61 |
| Ortho-toluidine | 66 | 41 | 38 | 66 | 37 | 41 | 45 | 61 | 50 | 42 | 47 | 49 |
| Glucose-oxidase | | | | | | | | | | | | |
| HClO | 44 | 43 | 23 | 52 | 22 | 30 | 24 | 50 | 47 | 37 | 37 | 38 |
| $Zn(OH)_2$ | 64 | 44 | 38 | 63 | 36 | 30 | 50 | 56 | 53 | 46 | 53 | 49 |
| Dextrostix | 40 | 40–65 | 40–65 | 40–65 | 40 | 0–40 | 40–65 | 90–120 | 40–65 | 40–65 | 40 | — |

The observed variations expressed as standard deviations given in Table 1 include both the analytical and the biological variations. In Table 3 are given the coefficients of variation (standard deviation calculated in per cent of the mean) for the different methods in normal newborn and in the test serum

*Table 3 Coefficients of variation for the concentration of whole blood glucose in normal newborn infants (biological and analytical variations) and in a commercial test serum (analytical variations)*

| Method | Normal newborn | Test serum |
|---|---|---|
| Hagedorn-Jensen | 19.5 | 5.1 |
| Ortho-toluidine | 20.6 | 4.5 |
| Glucose-oxidase | | |
| HClO | 23.4 | 6.1 |
| $Zn(OH)_2$ | 12.7 | 3.8 |

In T ble 4 the whole blood glucose concentration in normal newborn and infants with low birth weight estimated with Dextrostix are compared with the values obtained with the ortho-toluidine method.

*Table 4 Comparison between the whole blood glucose concentration determ ed with Dextrostix (abscissa) and the ortho-toluidine method (ordinate) in 60 fasting newborn infants 24–72 hours of age*

| Ortho-toluidine method (mg/100 ml) | Dextrostix (mg/100 ml) 0–39 | 40–64 | 65–89 | 90–120 |
|---|---|---|---|---|
| 65–89 | — | 4 | — | — |
| 40–64 | 6 | 36 | 3 | 1 |
| 0–39 | 5 | 6 | — | — |

Eleven of our observations with the ortho-toluidine method were below 40 mg/100 ml. Of these Dextrostix showed values below 40 mg/100 ml in 5 infants, 40–64 mg/100 ml in 6. Ten observations with Dextrostix were below 40 mg/100 ml. Five of the corresponding ortho-toluidine values were 40–64 mg/100 ml.

## Discussion

The discrepancies between the results obtained with the different methods must be due to either differences in the principle of the methods or in known or unknown interfering factors.

It is well known that the method of Hagedorn–Jensen includes several other reducing substances than glucose. It is assumed that the nonglucose reducing fraction counts for 10–30 mg glucose/100 ml [3 a]. This fraction shows individual and day to day variations [1]. In the erythrocytes about 5 times as much of the nonglucose reducing fraction is found as in the plasma [10]; obviously reducing substances in whole blood depend on the hematocrit value. The hematocrit in newborn infants varies according to the time of clamping of the cord and with age. According to the findings of Oh & Lind [8] the mean hematocrit value in our material is 65 %. The influence of the nonglucose reducing fraction will increase relatively with decreasing glucose concentration.

The ortho-toluidine method is specific for aldosugars. Lactose and mannose are normally not found in blood. In newborn infants on a milk diet, galactose could theoretically represent a source of error. As shown only negligeable amounts of galactose were found. Thus the ortho-toluidine method gives the true blood glucose level even in newborn infants.

The glucose-oxidase-peroxidase enzymes are influenced by numerous factors [3 b], hemolysis, bilirubin, glutathione, uric acid, cysteine, peroxides etc. The bilirubin and uric acid concentrations are considerably higher in newborn infants than in children and adults, and the mean glutathione concentration is approximately 30 % higher in the newborn period than later in childhood [11]. Glutathione, uric acid and vitamin C compete with the chromogen for the oxygen released from $H_2O_2$ by peroxidase, yielding false low glucose values. Peroxides not originating from the oxidation of glucose, together with other oxidants present, give unspecific oxidation of the chromogen causing false high values. Acid precipitating solutions, as perchloric acid, may release peroxides.

The values found when precipitating the proteins with perchloric acid were lower than when $Zn(OH)_2$ was applied. The discrepancy must be due to differences in the interfering factors in the glucose-oxidase-peroxidase system.

Unbuffered perchloric acid fails to remove glutathione [5, 12]. We have found no reports considering the effect on glutathione of perchloric acid buffered with glycine, but our results are similar to those found with precipitation of the proteins with unbuffered perchloric acid. The low glucose values estimated with the perchloric acid modification of the glucose-oxidase method are at least partly explained by the interference of glutathione [9]. The methodological problems with perchloric acid precipitation increase with decreasing glucose concentrations. Evaluation of the error introduced by these

factors is very difficult particularly at lower blood glucose concentrations. In the glucose-oxidase method with $Zn(OH)_2$ protein precipitation, however possible interfering factors are removed, and the method probably yields true blood glucose" values [5]

It is difficult to estimate the analytical variation when the concentration of glucose is in the order of 50 mg/100 ml. The calculations can be made from results obtained with aqueous 50 mg/100 ml glucose standard or a test serum. However none of these procedures yield conditions identical with whole blood in newborn infants. In order to get an approximate information the analytical variation was estimated by means of a test serum. From a clinical point of view all of the analytical methods employed give an adequate degree of precision (Table 3); but two of the methods fail with regard to accuracy The method of Hagedorn–Jensen gives values significantly higher than the true glucose The glucose-oxidase method when the precipitation of the proteins is carried out by means of perchloric acid gives values lower than the true blood glucose" Therefore these two methods cannot be recommended during the first period of life The correlation between Dextrostix and the ortho-toluidine method is satisfactory in the middle range of glucose values. The strip method failed, however in several cases with blood glucose concentration less than 40 mg/100 ml.

## Conclusion

Evaluation of the different methods demonstrates that the ortho-toluidine method and the glucose-oxidase method, the latter with $Zn(OH)_2$ protein precipitation, are the most accurate and reproduceable and hence the methods of choice in pediatrics Both of them yield results which are likely to be equal and reliable. The method of Hagedorn–Jensen and the glucose-oxidase method with precipitation of the proteins by means of perchloric acid are unreliable from a pediatric point of view In the method of Hagedorn–Jensen the unknown nonglucose reducing fraction plays a major part of the values obtained. After precipitation of the proteins by means of perchloric acid the enzymatic method yields very low and unreliable results. Dextrostix is not reliable as a screening test in the very low blood glucose concentrations.

## Summary

In the newborn infant special conditions prevail with regard to determination of the whole blood glucose concentration. The blood glucose is lower and the concentration of interfering substances is higher than in children and adults. Of five methods evaluated only the ortho-toluidine method and the glucose-oxidase method with $Zn(OH)_2$ deproteinisation were found to be reliable from a pediatric point of view The method of Hagedorn–Jensen, the glucose-oxidase method with precipitation of the proteins by means of buffered perchloric acid, and Dextrostix were found to be unreliable.

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# Regional lung function studied with $Xe^{133}$ in children with pneumonia

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Pneumonia is still a common disease especially in children and old people. The virus etiology is conspicuous. Pneumococcic pneumonia is, however nowadays perhaps as usual as before the antibiotic era [22], and the incidence of staphylococcic pneumonia is said to have risen in recent years [12].

It is well known that in pneumonia resolution is sometimes delayed and non complete. In Eaton-agent pneumonia as in virus pneumonia pulmonary dysfunction can exist for a long time after the acute stage [7, 9].

In order to achieve sensitive methods of evaluation of the pulmonary function bronchospirometry, lobar spirometry and methods for investigation of regional lung function with radioactive gases have been developed. Some of these methods were recently discussed in an international symposium [1]. Experience from bronchospirometry investigation in adults indicates that regional decrease in lung function may exist in spite of normal spirometry findings [1].

It would be of great interest to know to what extent pneumonia in childhood gives permanent impairment of regional lung function. As bronchospirometry and lobar spirometry are almost impossible to perform in children, methods using radioactive gases would be preferable for such studies. For that reason in a present study of pneumonia in children the regional lung function has been investigated with radioactive xenon ($Xe^{133}$). The experiences from this method in childhood have been good and a preliminary report will here be presented.

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## Method

### *For studying regional lung function*

#### *Principles*

The method is based on the fact that the gamma radiation from $Xe^{133}$ makes it possible to measure and localize $Xe^{133}$ present in the lungs with external detectors. $Xe^{133}$ is poorly soluble in blood and when injected intravenously 90 per cent will pass from the blood to the alveoli during the first circulation through the lungs; the amount arriving in each part of the lung being proportional to the blood flow to that part. The measurement of the relative distribution of $Xe^{133}$ injected intravenously is facilitated if the patient holds his breath for some 10 seconds after the injection, since $Xe^{133}$ in the alveoli is eliminated mainly through ventilation. When the patient starts to ventilate, the $Xe^{133}$ is exhaled and its disappearance rate from the lungs will depend on the efficiency of the alveolar ventilation. If $Xe^{133}$ is inhaled from a spirometer most of it will remain in

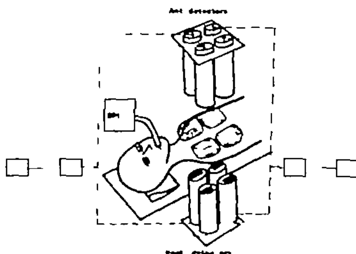


Fig. 1 Sketch in outline showing the arrangement of the 4 anterior and the 4 posterior detectors. The subject is in the recumbent position. The dark areas of the body are in the axillary plane. Their boundary lines represent the 50 % isocount line. R, recorder; SPIR, closed circuit spirometer

the alveoli (again because of its poor solubility in blood) and the amount arriving to each part of the lung with each breath is proportional t the ventilation of that part If the patient is rebreathing in a closed spirometer system, containing $Xe^{133}$ and an absorber of carbon dioxide to which oxygen is added so that volume is kept constant, a state will finally be reached where the concentration of Xe is the same in the spirometer and in the lungs. When this equilibrium has been reached, the radioactivity measured from each part of the lungs is proportional to the volume of that part. With proper technique it should therefore be possible to measure the relative distribution of perfusion, ventilation and lung volume with the aid of radioactive xenon.

*Technique*

A technique previously used for measuring regional lung function with Xe in adults (henceforth called radiospirometry) was used. Details of this technique are published elsewhere [.., 20]. The technique is based on investigations by Knipping *et al.* and Ball *et al.* [16, 5]. A brief description will, however be presented here.

The radioactivity from the chest is detected by 4 anterior and 4 opposing posterior detectors, coupled in pairs (Fig 1) The collimation is arranged so that the overlapping between the right and the left side is almost negligable.

*The patient is investigated in the recumbent position.* Before the investigation a plastic catheter is placed by the percutaneous technique in the femoral vein About 0.5 ml (0.5 mC) Xe is rapidly injected into the catheter immediately followed by 5–10 ml isotonic saline solution. Exceptionally for technical reasons, the brachial vein was used. After the injection, the patient holds his breath for 5–10 seconds. The children have previously been trained to hold their breath without straining Attempts were made to train the children to hold their breaths in resting expiratory level (FRC-position) but especially the younger ones had difficulty doing this without hanging the level of breathholding For that reason all the children were allowed t hold their breaths in tidal inspiration position.

When the radioactivity has returned t

Acknowledgement is due to S. E. Lindell and G Michaëlsson Department of Clinical Physiology Malmö General Hospital, where this radiospirometry method has been developed.

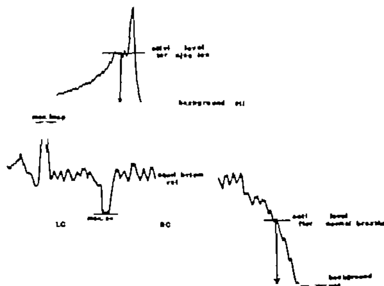


Fig. 2. Reproduction of records obtained at radiospirometry. The records are read from the right to the left. The designations are explained in the text.

background values the patient is connected to closed circuit spirometer and rebreathes a mixture of $Xe^{133}$ and air until equilibrium is reached. The patient is then told to do a maximal expiration and maximal inspiration.

*Calculations*

The radioactivity from the lungs is recorded in four channels, one for each of the four lung zones. The records from such a channel are shown in Fig. 2. In this curve p is the distance from background activity to the level of activity registered after intravenously injected $Xe^{133}$ during breathholding. This distance is used as measure of the perfusion (Q) of the lung section. The slope k is the decrease of activity when the patient starts t breathe and wash out the injected Xe. The slope can be analyzed mathematically and used for determination of the ventilation of the perfused alveoli [14]. The spike A is an artefact which ppears after the injection of Xe in a brachial vein. It ppears only in the curve representing the upper lung section of the same side as the injection, but with the help of the time markings the proper perfusion level can be found.

3 V (tidal volume) is the distance from background activity to the activity reached after the initial 3 normal breaths of Xe in the closed circuit system. FRC is estimated as the distance from background activity t the activity level at equilibrium. *Max. insp.* and *max exp.* are respectively the levels f activity at maximal inspiration and maximal expiration and the distance between them is taken as a measure of vital capacity (VC). The distance from max. insp. t background activity (TLC) and FRC are used as measurements of the pulmonary tissue covered by the two opposing detectors. All the measurements represent relative values.

The parameters of regional lung function and regional lung compartments are then expressed as the quotients of the values of the right lungfield to the values of the corresponding left lungfield, i.e. 3 V (right upper zone): 3 V (left upper zone) = the 1 ratio of upper zones. P (right upper

This means that decreased ventilation of the left lung will give high V ratio and



Fig. 3. Case 1. Initial roentgenogram of case 1 showing the small central parenchymal process in the left lower lobe

zone) $P$ (left upper zone) = the $Q$ ratio of upper zones.

If the distribution of pulmonary tissue among the 4 zones is assumed to be the same during the breathholding period after the injection of Xe as that after 3 breaths from the closed circuit spirometer the ratio of ventilation t perfusion can be calculated for each lung and each zone (i.e. $\frac{I_A}{Q}$ right upper zone =

$$= \left( \frac{\frac{3 \quad V_T \text{ (right upper zone)}}{\Sigma 3 \quad VT \text{ (all zones)}}}{\frac{P \text{ (right upper zone)}}{\Sigma P \text{ (all zones)}}} \right)$$

A more detailed analysis of the different parameters and their value as measure of the regional lung function in childhood will be given in other publications. In this report the $V_T$ ratio and the $Q$ ratio are used as ventilation and perfusion parameters.

*Radiation dose*

The tissue radiat on dose after injection and inhalation of Xe has been investigated carefully [17]. In the present study 1 mC Xe[133] (two injections) was administered intravenously and in the closed ircuit system the concentration f Xe[1] was 0.5 mC per liter In using these doses a complete investigation gives about the same gonadal dose as a routine chest x ray

## *Other lung function methods used*

Vital capacity (VC) forced expiratory volume in one second ($FEV_{1.0}$) and maximal voluntary ventilation in one minut (MVV) were studied with a modified Bern stein spirometer [6] Functional residual capacity (FRC) and lung clearance index (*LCI*, *Becklake's index*) were studied with N elimination technique using the Lilly $N_2$-meter as modified by Lundin [18 19]. All the investigations were made with the patient in the sitting position. The results are expressed in liters at BTPS and concerning FRC and FEV as per cent of TLC and VC respecti ely The expected normal values of static and dynamic lung volumes are derived from investigations by Bjure [6] and Engström *et al.* [11]. These values are represented in the tables as the mean values and $M$ – s.D

decreased ventilation of the right lung low $V_T$ ratio.



Fig. 4 Case 2. Initial roentgenogram of case 2, showing the diffuse parenchymal process in the apical part of the left lower lobe.

## Material

The material comprised 7 normal subjects and 3 children with pneumonia. The normal subjects were 9 10, 10, 11 13, 14 and 14 years old respectively To illustrat the method one case with a mild pneumonia of roentgenologically small extension, one case with a moderately severe pneumonia and one case with a roentgenologically extensive pneumonia were selected from a group of 11 children with pneumonia studied with the radiospirometry technique. In the hildren with pneumonia the first radiospirometry investigation was performed immediately after the disappearance of fever dyspnoe and chest pain. The histories of these children were as follows.

*Case 1* (J P 59 0? 10) was a 7 year-old boy with a small left-sided pneumococcal pneumonia (Fig. 3), in whom treatment with penicillin resulted in normalization of tem perature within 12 hours and a normal chest x-ray after 5 days.

*Case 2.* (B. L. 56 02 09) was a 10-year-old girl with a left-sided bacterial pneumonia and small pleural effusion (Fig. 4) in whom penicillin treatment resulted in normaliza tion of temperature within 12 hours. A chest roentgenogram was normal on the 13th day of the disease.

*Case 3* (J H. 59 03 10) was a 7 year-old boy with an extensive chiefly right-sided pneumonia of Eaton agent origin (Fig. 5). Tetracycline treatment resulted in normali



Fig. 5. Case 3 Initial roentgenogram of case 3, showing the extens parenchymal processes in the right upper and lower lobes

**TABLE 1** *Case 1 (small pneumococcic pneumonia in left lower lobe)*

Results of spirometry and radiospirometry

| Day after onset of disease … | | | 3 | 10 |
|---|---|---|---|---|
| Roentgenogram … | | | Normal (?) | Normal |
| *Spirometry* | *Normal values* | | | |
| | M 2 S.D | M | | |
| VC | 1.43 | 1.86 | 1.59 | 1.74 |
| FRC/TLC, % | 31 | 41 | 55 | 49 |
| FEV /VC, % | 74 | 85 | 86 | 84 |
| MVV | 45 | 63 | 56 | 58 |
| LCI | — | — | 6.0 | 6.0 |
| *Radiospirometry* | *Normal range* | | | |
| $\dot V_T$ ratio upper | 1.01–1.23 | | 1.13 | 1.00 |
| lower | 0.99–1.30 | | 1.30 | 1.01 |
| $\dot Q$ ratio upper | 1.01–1.28 | | 1.19 | 1.16 |
| lower | 0.91–1.30 | | 1.26 | 1.01 |
| $\dot V_A/\dot Q$ (left lung) | | | | |
| upper | 0.85–1.05 | | 1.13 | 1.14 |
| lower | 0.90–1.12 | | 0.77 | 0.93 |

xation of temperature within 24 hours. A roentgenogram after 8 days of treatment revealed remnant of the pneumonic process.

## Results

The radiospirometry results obtained in the 7 normal children are presented as the range in Tables 1, 2 and 3. These values agree very closely with the results from investigation of a large number of normal adults [20]. The results of the lung function studies obtained in case 1 is presented in Table 1. On day 3 of the disease the results of ordinary spirometry and radiospirometry were on the whole normal. However, the FRC/TLC quotient (55 %) was too high and the ratio of ventilation to perfusion of the affected lung field (0.77) slightly too low, which values were normalized one week later on.

Table 2 gives the results of lung function studies in case 2. The first studies were performed on day 7 of the disease when a roentgenogram showed sparse remnants of the parenchymal process and the spirometry values were abnormal.

**TABLE 2.** *Case 2 (left-sided bacterial pleuropneumonia)*

Results of spirometry and radiospirometry

| Day after onset of disease | | | 6 | 14 | 60 |
|---|---|---|---|---|---|
| Roentgenogram … | | | Thin process in basal part of left lower lobe | Normal | Normal |
| *Spirometry* | *Normal values* | | | | |
| | M 2 S.D | M | | | |
| VC | 2.32 | 2.89 | 2.–4 | 2.37 | 2.40 |
| FRC/TLC, % | 31 | 41 | | 48 | 37 |
| FEV /VC, % | 80 | 89 | 87 | 84 | 77 |
| MVV | 78 | 101 | 60 | 72 | 85 |
| LCI | — | — | | 8.1 | 11.4 |
| *Radiospirometry* | *Normal range* | | | | |
| $\dot V$ ratio upper | 1.01–1.23 | | 1.74 | 0.99 | 1.10 |
| lower | 0.99–1.30 | | 1.76 | 1.16 | 1.54 |
| $\dot Q$ ratio upper | 1.01–1.28 | | 1.54 | 1.37 | 1.17 |
| lower | 0.91–1.30 | | 1.29 | 1.23 | 1.30 |
| $\dot V_A/\dot Q$ (left lung) | | | | | |
| upper | 0.85–1.05 | | 0.85 | 1.00 | 0.83 |
| lower | 0.90–1.1 | | 0.88 | 1.11 | 0.07 |

TABLE 3 *Case 3 (chiefly right-sided, Eaton agent pneumonia)*

Results of spirometry and radiospirometry

| Day after onset of disease — Roentgenogram — | | | 7 Extensive process in right lung | 14 Remnants of initial process | 40 Normal |
|---|---|---|---|---|---|
| *Spirometry* | Normal values $M \pm$ S.D. | $M$ | | | |
| VC | 1.62 | 2.11 | 1.08 | 1.66 | 05 |
| FRC/TLC, % | 31 | 41 | 67 | 55 | 47 |
| $FEV_{1.0}$/VC, % | 74 | 85 | 78 | 81 | 79 |
| MVV | 52 | 73 | 30 | 48 | 53 |
| LCI | — | — | 10.9 | 7.6 | 6.6 |
| *Radiospirometry* | Normal range | | | | |
| $\dot{V}$ ratio upper | 1.01–1.23 | | 0.19 | 0.61 | 0.90 |
| lower | 0.99–1.30 | | 0.09 | 0.74 | 0.86 |
| $\dot{Q}$ ratio upper | 1.01–1.28 | | 0.40 | 0.64 | 0.87 |
| lower | 0.91–1.30 | | 0.41 | 0.76 | 0.93 |
| $\dot{V}/\dot{Q}$ (right lung) | | | | | |
| upper | 0.78–1.17 | | 0.64 | 0.94 | 1.38 |
| lower | 0.90–1.12 | | 0.24 | 0.91 | 0.82 |

Radiospirometry revealed impaired ventilation of both zones ($\dot{V}$ ratio = 1.74 & 1.0) and impaired perfusion of the upper zone ($\dot{Q}$ ratio = 1.54) of the affected side: the ventilation was more reduced than perfusion. On the 14th day of the disease a roentgenogram was normal as were the results of ordinary spirometry except the MVV value. At a follow up investigation months after the onset of the disease when she was in good health with normal chest x ray, ESR and blood values, lung function studies were repeated. Spirometry revealed a slightly decreased FEV % (77) and a probably abnormal LCI value (11.4). Radiospirometry disclosed an obvious decrease of ventilation in the left lower zone ($\dot{V}$ ratio = 1.54) but perfusion and the ratio of ventilation to perfusion were normal.

Table 3 gives the results of lung function studies performed in case 3. The first studies were performed one day after the initial chest x ray investigation (Fig. 5). Spirometry showed values below the lower normal limit and radiospirometry revealed greatly reduced ventilation and considerably reduced perfusion of the affected side. On day 14 of the disease when a roentgenogram showed considerable resolution, ordinary spirometry revealed a low MVV value and a high FRC/TLC quotient. Radiospirometry disclosed a decrease of ventilation and perfusion. At a follow up investigation 1½ months after the onset of the disease he was in good health with normal ESR and blood values. A roentgenogram was normal as were the results of ordinary spirometry. Radiospirometry however revealed slightly reduced ventilation of the right lower zone ($\dot{V}$ ratio = 0.86) and slightly impaired perfusion of the right upper zone ($\dot{Q}$-ratio = 0.87). The ratio of ventilation to perfusion was abnormal for the right upper zone (1.38).

## Discussion

In pneumonia a true etiological diagnosis is difficult to achieve. Concerning the here reported patients the first one had probably a pneumococcic pneumonia, the second one a bacterial pleuropneumonia and the third one a pneumonia of Eaton agent origin.

In the acute stage VC and MVV were reduced in _ cases. These spirometric measurements have in other studies been found to be decreased in bacterial pneumonia as well as in virus pneumonia and above all the MVV value seems to have been affected [10 15]. The FEV % value was in all 3 cases normal indicating lack of bronchospasm, which is in agreement with other investigations [10 15]. In varicella pneumonia, however obstructive ventilatory insufficiency has been found [9].

The FRC/TLC % measurement was increased in the acute or post-acute stage in 2 cases indicating increased resting expiratory level. In the acute stage of pneumonia the helium mixing time has been found to be increased [10]. In the present study the lung clearance index (LCI) decreased with clinical improvement in two of three cases.

At the follow up investigations all the mentioned parameters were normal except in case 2 where both the slightly decreased FEV % value and the high LCI value may indicate a subclinical bronchobstruction.

In the immediately post-acute stage of the pneumonia the results of radiospirometry are in good agreement with the roentgenological extension of the pneumonic process and the spirometry results. The pattern of changes seemed to be the same in all three cases; in the pneumonic area the ventilation was more reduced than the perfusion which is evident from the ventilation and perfusion parameters in two of the subjects as well as from the decreased ratio of ventilation to perfusion in the 3 subjects. It has been stated earlier that the arterial hypoxia seen in acute pneumonia is due to shunting of blood through the badly ventilated pneumonic tissue and to inequality of ventilation to perfusion [4 10]. The present results indicate that this might be true. Bronchospirometric investigations of adults with lobar pneumonia, however disclosed more reduced perfusion than ventilation [3].

During the course of the disease a good agreement was on the whole established between the results of spirometry and radiospirometry.

Of interest is that the follow up investigation disclosed 2 of the patients to have slight pulmonary dysfunction in spite of clinically good condition and normal chest x ray. In case 2 (bacterial pleuropneumonia) the regional ventilation was affected, possibly as a result of scarring of the pleura, and in case 3 (Eaton agent pneumonia) ventilation as well as perfusion were slightly reduced but not to the same degree resulting in an inequality of ventilation to perfusion.

In previous investigations in adults impairment of pulmonary function (abnormal anatomic shunts, decreased pulmonary diffusing capacity and reduced spirometric measurements) has also been found long time after the acute stage of pneumonia [7 9 10 23]. The significance of this with regard to the future health of the patient is difficult to evaluate but clinical reports indicate that an acute

pneumonia is often the origin of future chest disease [13]

With the present technique cooperation of the patient is necessary to about the same degree as in ordinary spirometry but with altered technique and injection of $Xe^{133}$ during normal breathing children younger than 6 years can probably be investigated.[1] Our experience indicates that radiospirometry is a good method for studying regional lung function in children.

## Summary

A method for measurement of regional lung function (radiospirometry) in children is presented. With radioactive Xenon the regional pulmonary ventilation, perfusion and the ratio of ventilation to perfusion could be estimated. The radiation dose used in this study was small. The method has been used for an investigation of the lung function in children with pneumonia. The results obtained in three cases of pneumonia of different etiology and extension are reported. In the immediately post-acute stage the ventilation of the pneumatic tissue seemed to be more affected than perfusion. At follow-up investigations 2 of the cases had abnormal lung function in spite of normal chest x ray

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# Early Neonatal Jaundice and Hyperbilirubinaemia and Their Relation to ABO Incompatibility

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Jaundice appearing in a newborn during the first 24 hours of life is considered to be unphysiologic and frequently related to haemolytic disease. This symptom is well known, e.g. in children with haemolytic disease caused by maternal Rh immunization, and is a valuable sign in detecting cases of ABO immunization. Early jaundice does not, however necessarily lead to clinically important hyperbilirubinaemia, and very high levels of bilirubin may on the other hand develop in babies who appear jaundiced later than 24 hours after birth. The purpose of the present investigation was to study the value of this symptom in predicting the development of hyperbilirubinaemia, necessitating special observation and treatment. Additionally an attempt was made to evaluate the role of ABO incompatibility as a factor causing early neonatal jaundice

## Material and Methods

The series was collected during a 15-month period, between March 1961 and June 1962. The total number of babies born during that period, in the Women Clinic, was 6549 excluding stillbirths, newborns with birthweight less than 2500 g, and those born to Rh immunized mothers or transferred to pediatric wards for other reasons than jaundice during the first day of life.

From this material, all babies found by the nursing staff to be recognizably jaundiced within 24 hours after birth were subjected to closer study From the cases to be studied a venous sample was drawn in heparinized tubes the weekday morning after the jaundice had been noticed. The following laboratory tests were performed by standard methods: haemoglobin (as haemoglobin cyanide) microhaematocrit, reticulocyte count, ABO group, Rh type and direct Coombs test. Direct and indirect serum bilirubin was determined by a modification of the Malloy Evelyn method according to Gellis & Hsia [3].

The maternal ABO and Rh group was determined simultaneously and the mother serum was screened for irregular antibodies.

All babies with indications for closer observation or special treatment were transferred to the Children Hospital, University of Helsinki.

In addition to the cases jaundiced before 24 hours of age there were during the period of investigation, 53 babies who developed marked jaundice after the 24 hours limit. These cases were subjected to the same investigations as the early neonatal jaundice group. Of the latter group 18 were transferred to the Children Clinic for observation, and exchange transfusions were carried out when necessary

TABLE 1 *Percentage of mother–child combinations in the general population in Finland.*

| Group of mother | Children groups O | A | B | AB | Incompatible |
|---|---|---|---|---|---|
| O | 18.5 | 9.5 | 4.5 | 0 | 14.0 |
| A | 9.5 | 16.4 | — | 3.5 | 6.7 |
| B | 4.5 | .3 | 3.1 | .8 | 3.1 |
| AB | 0 | 3.5 | .8 | 1.7 | 0 |
| | | | | Total | 24.8 |

Based on gene frequencies A-0.290; B-0.138; O-0.57 [10].

## Results

Altogether 163 of the 6549 babies were reported to be jaundiced before the age of 24 hours. This gives a frequency of 2.49 per cent or 1 out of 40 fullterm babies.

It must be pointed out that the material, owing to the very subjective method of selection, is bound to include children with low bilirubin values who were erroneously recognized as jaundiced before 24 hours of age It is on the other hand likely that there were undetected cases of early jaundice and that a part of these were included in the group of late hyperbilirubinaemia.

The birthweights showed no obvious diff rence from normal distribution.

The sex incidence was 48.1 per cent boys in the combined series (5*.3 per cent and 41.5 per cent among ABO compatible and incompatible respectively)

There was no clinical evidence of viral or bacterial infection in any of the cases. All the infants were in good general condition.

During the study 1 mg doses of vitamin K were given in certain wards to all babies and in other wards to some but not all, according to liberal indications. It appeared obvious from the incidence of cases in these groups that vitamin K in the doses used, was not a factor contributing to early jaundice or hyperbilirubinaemia.

The feeding of newborns was uniform throughout the hospital. Glucose solution was given from the age of 12 hours and breast feeding was started at 24 hours of age.

*Mother/child blood group combinations*

The distribution of mother child combinations in the general population can be calculated with known values for the gene frequencies according to Schiff [9] and Wiener [12]. Calculated percentages for the Finnish population are shown in Table 1

Blood group combinations for the 24 hr material are given in Table 2. The ABO incompatibility rate is 63 of 163 children. or 38.7 per cent. The corresponding figure in the general population is 24.8 per cent The number of ABO incompatible children in the group of late hyperbilirubinaemia was 21 of 53 or nearly 40 per cent

Using the percentages in Table 1 the expected blood group combinations for the combined series were calculated as follows. The total number of ABO compatible

TABLE 2. *ABO group of mothers and children in the 24 hr series*

| Group of mother | Children groups O | A | B | AB | Total | Incompatible |
|---|---|---|---|---|---|---|
| O | 26 | 30 | 14 | — | 70 | 44 |
| A | 16 | 39 | 3 | 5 | 63 | 10 |
| B | 8 | 3 | 6 | 6 | 23 | 9 |
| *AB* | — | 7 | 4 | 1 | 12 | — |
| Total | 44 | 78 | 29 | 13 | 163 | 63 |

TABLE 3 *Expected (see text) and observed distribution of blood groups in the combined materials*

| Group of mother | Children groups O Ex pected | O Ob-served | A Ex pected | A Ob-served | B Ex pected | B Ob-served | AB Ex pected | AB Ob-served | ABO incompatible Ex pected | ABO incompatible Ob-served |
|---|---|---|---|---|---|---|---|---|---|---|
| O | 32.9 | 32 | 16.7 | 37 | 7.9 | 21 | 0 | 0 | 4.6 | 58 |
| A | 16.7 | 13 | 46.8 | 53 | 4.0 | 6 | 6.2 | 7 | 10 2 | 13 |
| B | 7.9 | 9 | 4 0 | 5 | 14.3 | 7 | 4.9 | 8 | 8.9 | 13 |
| AB | 0 | 0 | 6.2 | 8 | 4.9 | 6 | 3.0 | 2 | — | — |
| | | | | | | | | | 41.7 | 84 |

babies of 132 should represent 75_ per cent of an unselected material. The number of ABO incompatible babies should accordingly be (13_/75.2) × 4.8, i.e. 44 giving an expected total number of 13* + 44 = 176. The expected and the observed number of cases are shown in Table 3

The excess of ABO incompatible babies is accordingly 40 which means in effect that 40 cases of 6549 belong to the series because of ABO incompatibility an incidence of 1 164 If this incidence is calculated from the estimated number of ABO incompatible babies only again using the general population frequencies (40 out of 1024) it is 1 40 or _5 per cent.

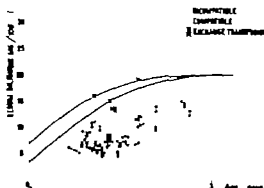
It is accepted that ABO haemolytic disease is more frequently seen in incompatible babies with an O mother than in other blood group combinations. The majority of excess incompatible cases in this material belong to group O mothers as seen in Table 3. The same fact is evident when comparing the number of blood group combinations "Mother O/Child A or B with the combination Mother A or B/Child O There were 58 babies in the former and 22 in the latter group. In an unselected material these numbers should be equal.

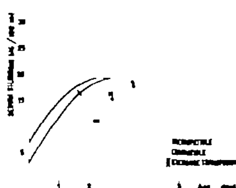
If group A and B in infants were equally liable to ABO haemolytic disease the ratio of affected infants should be that of the gene frequencies of A and B [7] With the high B frequency in Finland the ratio is much lower than e.g. in England, where it is 0.290/0.138 or (° 1) The proportion of A and B children of group O mothers in the present material is 1.8 1

As stated before, all cases of Rh immunization were excluded from the series. In all infants the direct Coombs test was negative nor were irregular antibodies found by screening the maternal serum. The unmodified direct antiglobulin test used in this study gives only occasionally positive results in cases with ABO haemolytic disease

*Haemoglobin concentration and reticulocyte count*

The haemoglobin values are influenced in two ways during the first days of life The shift of plasma from the circulation leads to haemoconcentration in practically all infants. Increased red cell destruction may on the other hand cause decreased


Fig. 1 Serum bilirubin values in the 24 hr series.


Fig. 2. Serum bilirubin values in the series of late hyperbilirubinaemia.

haemoglobin values not only in the cord blood, but also during the first day of life [6]

During the present investigation no cord blood samples were collected. The age of the babies varied considerably at the *time of sampling, and differences between* individual haemoglobin values are therefore difficult to evaluate The mean for the haemoglobin values in the total series was 18.3 g/100 ml, with a minimum value of 12.8 g/100 ml and only 5 cases below 15 g/100 ml.

Reticulocyte counts averaged 4.1 per cent in incompatible babies and 3 0 per cent in compatible. Individual values ranged from 1 to 8 per cent. Values above 5.5 per cent were encountered almost exclusively in incompatible cases.

*Serum bilirubin*

Indirect bilirubin values are shown in Figs. 1 and 2 *ABO incompatible* children tend to have higher concentrations of bilirubin than compatible ones. This is seen more clearly if the rise of bilirubin is calculated in individual cases (bilirubin in mg is divided by age in hours, assuming that the cord value is zero). Fig 3 illustrated that among cases with a bilirubin rise of more than 0.3 mg/hr there is an excess of incompatible children, and extremely high values are seen only in incompatible babies. Inclusion of late hyperbilirubinaemia cases does not cause any substantial alternation *in the percentile distribution of cases in* Fig 3

The rise of bilirubin as a function of time was also compared with such factors as parity labor gestational age, birth weight and placental weight. None of these showed any significant effect on the rise of bilirubin values.

*Exchange transfusion*

Exchange transfusion was performed in 21 cases altogether (Table 4) with fresh,

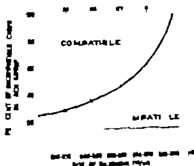


Fig. 3. Rise of bilirubin in ABO compatible and incompatible cases.

Table 4 *Number of exchange transfusions in the different groups*

| | Jaundiced (24 hr/later) | Incompatible (24 hr/later) | Compatible (24 hr/later) |
|---|---|---|---|
| Exchange transfused | 13/8 | 11/5 | 2/3 |
| Not treated | 150/45 | 52/16 | 98/29 |
| Total | 163/53 | 63/21 | 100/3* |
| | 216 | 84 | 132 |

heparinized compatible blood in ABO compatible babies and with O blood in cases of ABO incompatibility Although the indication for treatment was mainly the actual level of serum bilirubin and the calculated risk of reaching bilirubin concentrations well over 20 mg/100 ml, the presence of ABO incompatibility may to some extent influence the decision. This is partly due to the fact that exchange transfusion is considered to be causal therapy in ABO incompatibility the removal of bilirubin being the only purpose in compatible cases. It is on the other hand evident that the proportionally high number of incompatible cases in the treated group depends mainly on the fact that high bilirubin values developed in these babies.

It should also be noted that early jaundice was discovered in 75 per cent of the cases where exchange transfusion was considered indicated.

## Discussion

Recognition of early jaundice with the naked eye can never be completely reliable: it probably varies even within the same hospital for many reasons. This is reflected in the present series, in the number of cases where the actual bilirubin concentration was very low certainly lower than in many normal cases without visible jaundice The relatively high number of babies who later developed severe jaundice, estimated again subjectively makes it, on the other hand, probable that part of them were also jaundiced before the age of 24 hours but were undetected. Although the present series apparently includes cases wrongly diagnosed as early neonatal jaundice it is probable that very few babies with this symptom escaped the investigation. This applies to both groups, early jaundice, or later hyperbilirubinaemia For reasons given above, the incidence of early jaundice 1 40 or if both groups are combined, 216 out of 6549 i.e 1 30 is too high and cannot be compared with figures obtained from series in which more reliable methods of detection were used. If all cases with a bilirubin rise of less than 0 2 mg per hour are excluded, there remain in the combined series 145 cases, giving an incidence of 1 45 The corresponding figures in the early jaundice group are 110 and 1 60 These are of the same magnitude as the figures reported by Valentine [11] and by Cunningham [1] who found 14 and 4 cases respectively among 1000 newborn babies, but much higher than the incidence of 1 140 found by Hsia & Gellis [6] or 1 180 reported by Hallbrecht [4] Although no definite conclusions regarding the incidence of neonatal hyperbilirubin-

aemia are justified, the early jaundice was in the present series a valuable clinical symptom The majority of cases which developed hyperbilirubinaemia and later underwent exchange transfusion were thus detected during the first day of life

Much more interesting than the incidence of early jaundice is the role of possible aetiological factors in the development of hyperbilirubinaemia. The cases of ABO incompatible infants, 40 out of 6540 giving an incidence of 1 160 should be independent of the method of detection In other words, the presence of wrongly diagnosed cases should not influence this excess. This is illustrated by leaving out cases with a rise of less than 0.2 mg per hour There remain 145 cases 63 incompatible and 82 compatible. A calculation *similar to that presented on page 478 shows* that the excess is almost the same, 38.

The excess of ABO incompatible infants is mainly caused by A or B babies with O mothers (Table 3) and corresponds with the wellknown clinical observation of more severe haemolysis in these blood group combinations than in ABO incompatibility with A or B mothers. The role of ABO incompatibility as an aetiological factor is further seen in Fig 3 where most of the cases with a rapid rise of bilirubin concentration belong to the incompatibility group In the group with a slow bilirubin accumulation, the frequency of ABO incompatibility differs only slightly from the frequency in a random population.

The well-known mechanism of haemolytic disease caused by Rh immunization is probably the reason why ABO haemolytic disease has been generally accepted as similar clinically It seems for various reasons however improbable that shortening of the life span of ABO incompatible red cells in the foetus and the newborn should be restricted to cases with clinical manifestations of haemolytic disease. The wide individual variations in haemoglobin concentration, reticulocyte count and other signs of erythropoietic activity make it difficult and in most cases, impossible to discover cases with shortened red cell survival. The accumulation of bilirubin or in extreme cases, the development of hyperbilirubinaemia is influenced by two opposing factors breakdown of haemoglobin as a source of bilirubin, and the capacity of the liver to conjugate and excrete bilirubin. This is illustrated by the following calculation.

It seems probably that the survival of red cells in ABO compatible newborn babies is uniform, with only a few exceptions The life span seems to be shorter than that of adult red cells, or about 70 days [2 8] Accordingly the following calculation is based on a daily breakdown of 1 5 per cent of the cells, i e. this amount of haemoglobin is daily metabolized into bilirubin. Assuming the haemoglobin content is 20 g per 100 ml of blood 0,3 g of haemoglobin is liberated in 24 hours This corresponds to 10.2 mg bilirubin per 100 ml of blood, if it was accumulated in the plasma only [13] If the haematocrit is 50 per cent, this would give a bilirubin concentration of 20.5 mg/100 ml Half of the bilirubin, however will move to the interstitial plasma compartment, of the same magnitude as the intravascular plasma compartment which again leaves in the circulation 10.2 mg bilirubin per 100 ml A total excretion of this amount

would mean that the liver must conjugate approximately 0.4 mg bilirubin per hour in other words, a total lack of conjugation would result in a maximal rise of 0.4 mg bilirubin per 100 ml per hour. With a red cell life span of 100 days instead of 67 as used in the calculation above, the corresponding figure would be about 0.3 mg bilirubin per 100 ml per hour.

The actual rise of plasma bilirubin is further influenced by two additional mechanisms. The conjugating capacity of the liver is improved and finally normalized in the first week of life. It might also be dependent on the actual level of bilirubin in the plasma, i.e. the total actual capacity is not used before elevated bilirubin levels are reached. The rise of bilirubin concentration is less than 0.1 mg/100 ml per hour in the vast majority of newborn babies. This shows that the capacity to conjugate bilirubin in most instances is at least 0.3–0.5 mg/100 ml per hour and probably more, as a rule.

This argument supports the idea presented by Mollison [7] that a shortened life span of ABO incompatible red cells is much more common than the incidence of hyperbilirubinaemia observed in ABO incompatible newborn infants. It also means that early jaundice and hyperbilirubinaemia are seen only in severe cases of ABO incompatibility where the haemolytic process reaches a degree seen in Rh haemolytic disease, or in cases where the infant, by coincidence, has simultaneously a poor capacity to conjugate bilirubin. On the other hand, jaundice develops even with a normal erythrocyte life span, as in ABO compatible babies, if there is a discrepancy between the bilirubin produced and the excretory capacity.

## Summary

A series of 6549 babies in the Women's Clinic University of Helsinki was studied with reference to early (before 24 hours of age) jaundice and hyperbilirubinaemia and its relationship to ABO incompatibility. Of the babies 163 or 1: 40 were found to be jaundiced before 24 hours of age. 63 babies developed severe jaundice later and were included in the total material. The incidence of ABO incompatible babies was higher than the expected value in the general population (83 against 44). Of these excess cases the majority belonged to blood group combination mother O child A or B. The rise of bilirubin per hour was higher among ABO incompatible cases than among ABO compatible. The conditions leading to hyperbilirubinaemia in newborn are discussed. It is concluded that ABO incompatibility is an important factor causing early jaundice of hyperbilirubinaemia. 5 per cent of cases requiring exchange transfusion developed early jaundice. It seems, on the other hand, probable that children with a good excretory capacity of bilirubin seldom develop jaundice.

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# Three New Cases of Histidinemia

## *Clinical and Biochemical Data*

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Since the first publications by Ghadimi et al. [10 11] 15 children with histidinemia have been described [1 2, 5 7 8 13 1 25]. In this article three further cases of histidinemia are reported.

## Case Reports

Case 1

A girl (M t B ) born July 7th, 1957 was the third child of healthy parents. The first child, a boy was still-born; the second one a girl, is normal. The patient was born after a normal pregnancy She weighed 3600 g The child did not breathe spontaneously and was cyanotic. After repeated warm and cold baths she started to breathe She was mildly jaundiced.

Her development physically and mentally was slow At the age of 10 months she was able to sit alone One month later she had a gastro-intestinal disorder Before she could sit again she was 18 months old. The parents felt that the development had slowed down more and more after the age of 10 months. When the child as 4 years old she was ble to speak some words although unable to make sentences She said L instead of R, and even now she is still very difficult to understand. The child was slow compared ith other children of the same age The words esterday and "tomorrow meant nothing to her She preferred to play with younger children. The child never had convulsions, she always complained of tiredness was mildly anorexic and vomited regularly

Up until the age of 7 years she only took fluid foods. On physical examination (Aug 9th, 1965) when the child was 8 years, her height was 137 cm, her weight .5.2 kg. She had a funnel-shaped chest, but no further bnormalities. The mental retardation of the child gave rise to aminoacid analysis of the urine A pronounced histidinuria was an indication for further biochemical investigation.

Case 2.

A girl (A B ) born April 4th, 1954 was the first child of healthy parents. In the same famil are a normal daughter and son. There is no consanguinity of the parents. The child was born after normal pregnancy labor and delivery; she weighed 3000 g. The neonatal course was uneventful. The child was breastfed till the age of 9 months, and gained eight normally Her development was as follows, she laughed at six weeks. She could sit before the age of 1 year She w lked alone at 18 months. The child was toilet trained at 4 ears during da time and 1 year later als at night When the child was 1½ years she had convulsion, and again some months later

On ph sical examination (Aug. 24th, 1964) hen the hild was 10 years old, her height as 144 cm, her weight 31 6 kg. She had tall slender stature and red hair She was t persil fraid of new situations Except

for the appearance of pubic hair there were no other particulars on examination. The child was referred to us because she had a positive ferric chloride test of the urine.

### Case 3

A girl (C. G.) born June 6th 1964, was the 5th child of healthy parents. After the 4th child the mother had 5 abortions, the first one after 3 months pregnancy the other four after months. There was no consanguinity of the parents. From the beginning of the pregnancy the mother was on a salt restricted diet, there were complaints of nausea, and in the 3rd month, the mother suffered from "influenza". The delivery was uneventful; the birthweight was 2800 g. From the beginning the child would not drink, developed a grave jaundice and was drowsy. At the 4th day of life she was admitted to a hospital elsewhere. The baby was inactive and did not cry forcibly. She was tube-fed. The cause for this grave jaundice was unknown. There was no blood group incompatibility nor signs of hypothyroidism. Galactosemia was excluded by urine examination. During hospital admission elsewhere the child would not drink actively. She did not cry nor react on sounds. At the age of 4 months the ferric chloride test of the urine was positive and a phenylalanine-restricted diet was given. Amino acid analysis of blood and urine indicated that the child had histidinemia instead of phenylketonuria.

On December 1*th, 1964, the child was hospitalized in the Wilhelmina Children's Hospital. She was 6 months old, her height 62.5 cm and her weight was 5540 g. She had high forehead and a large anterior fontanel, the head circumference was 42 cm. At physical examination she exhibited hypertelorism, epicanthus and a big umbilical hernia without any further abnormalities. There were no specific neurological symptoms and signs. Laboratory studies showed a normal blood count with the exception of a mild anaemia (hemoglobin 9.2 g/100 ml), normal values for calcium, phosphorus, phosphatase, urea, creatinine, thymol turbidity test, blood protein, albumin globulin ratio and immuno-electrophoresis.

By routine examination of the urine no abnormalities were found. By physical, neurological and psychological examination a slight retardation was found at the age of 8 months. Her mental age was approximately 6 months. It was not until the child was one year old that we were able to prescribe a histidine-restricted diet. With the exception of a short period just before therapy was started (and also of the time before admittance) she had a diet restricted in protein. On March 3rd, when the child was 9 months old, she started to drink some food actively and 11 days later she took the whole feed.

## Methods

*Screening of amino acids in urine* [4] was accomplished by 2-dimensional paper chromatography on sheets of Whatman no 1 filterpaper 23 × 23 cm ascending method.

System I lutidine pyridine butanol water (8 7 7 13). System II. 800 g phenol + 200 g water + 1 ml $NH_3$ 10 % + 5 mg oxyquinoline. Ninhydrin spray. Preliminary oxydation of SH-amino acids by $H_2O_2$ 30 %.

The urinary aminoacids (and imidazoles) were isolated by a standard method consisting of (1) absorption by Dowex 50 × 8 cation exchanger (50–100 mesh, $SO_3H$ form) ( ) elution by 2 n ammonia; (3) evaporation of the eluate to dryness in a rotating vacuum evaporator at 40 C and dissolution of the residue in one tenth of the original volume. An amount of this concentrate, corresponding to mg of total nitrogen (Kjeldahl method) was applied on the chromatogram. The concentrate was also used for the estimation of α amino-N [18].

TABLE I *Histidine in serum and urine of the younger patient C G and control baby E (girl, 7/8-65 7 0 kg creatinine excretion of the same magn tude as C G).*

| | Date of analysis | Histidine intake (mg/kg/24 hr) | Fasting serum histidine (mg/100 ml) | Histidine in urine (mg/g creatinine) |
|---|---|---|---|---|
| Patient C. G | 27/10-64 | 125 | 18.0 | 2077 |
| | 20/12-64 | 59 | 12.8 | 1409 |
| | 26/1-65 | 59 | 12.6 | 1630 |
| | 14/6-65 | 115 | 17.0 | 1695 |
| Control baby E. | 31/3-66 | 95 | 1 49 | 371 |

*Quantitative determinations of histidine and other amino acids* in serum, cerebrospinal fluid and urine were performed by quantit tive ion exchange chromatography W used the Technicon amino acid analyzer; for analyses of serum the 12½ hour method [22] and deproteination by ultrafiltration with centrifugal force [23] for urine analyses the 22 hour standard method. Interference of 3-methylhistidine with histidine was neglected.

*Imidazole compounds.* Imidazolelactic acid hydrochloride was synthetized according to Pyman [19]. The HCl-salt was converted to the NH -salt by absorption on Dowex 50 and elution by 2n ammonia. By treating the $NH_4$-salt by an equivalent amount of Amberlit IRC 50 we obtained chromatographically pure free imidazolelactic acid M 156,2, mp 217°C with an overall yield of 72% in reference t -Cl-β-imidazolepropionic acid, [9]. The latter compound was synthetized. Imidazoleacetic acid hydrochloride was purchased from Koch-Light Colnbrook; N acetylhistidine $H_2O$ and imidazolepyruvic acid hydrochloride ½ AcOH from Calbiochem., Los Angeles.

For 2-dimensional paperchromatography of urinary histidine metabolites 0 05 ml of amino acid concentrate was pplicated on a 23 23 cm sheet of Whatman paper no. 1

System I. isopropanol ammonia 5 % (4 1). System II butanol acetic acid water (4 1 1). Spray diazotized sulfanilic acid—$Na_2CO$ 10 %. Imidazolepyruvic acid is lost for the greater part.

Quantitative determinations of imidazolelactic acid, imidazoleacetic acid and N acetylhistidine were performed by elution of spots from the same 2-dimensional chromatogram (multiple development was pplied to obtain complet separation) and subsequent colorimetry t 495 mμ.

For one determination of imidazolelactic acid, imidazoleacetic acid and N-acetyl histidine 3 different chromatograms were run, starting from 3 different volumes of concentrat e.g. 0.01 0.0* and 0.03 ml. From plotting of extinctions versus micrograms straight lines result. From the slopes of these lines the concentrations of the imidazoles can be derived. For calibration a single standard solution containing the 3 compounds is run through the procedure.

Standard deviations were calculated from the xtinctions, obtained from standard solutions on six different days. Sw (as % of means) appeared to be 5.9 %, 5.8 % and 11 4 % for respectively imidazolelactic acid, imidazoleacetic acid and N-acetylhistidine

## Biochemical D ta

*Serum histid ne* All three patients have a significantly elevated serum histidine concentration, as can be read from Tables I and With the method used 1 4 mg/100 ml (s D 0 63) has been found for the normal fasting serum histidine of children + adults The serum level of the youngest

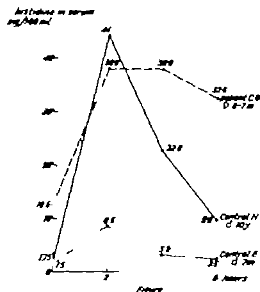


Fig. 1. Rise in serum histidine after oral administration of l-histidine, 270 mg/kg body weight.

patient C. G. is higher than that of the older patients, possibly as a result of a higher histidine intake per kg. In patients with a metabolic defect a relation between intake and serum concentration can be expected.

In Fig. 1 the rise in serum histidine after oral administration of l-histidine $OH_2O$ OHCl, 270 mg/kg is given.

Patient C. G. is compared with two controls E. and H. Serum concentrations of C. G. and control H. rose to a high level.

In H. there was a rapid decrease after 4 and 6 hours, however in C. G. the high level was sustained, after 6 hours there was only a small fall. This sustained elevation of serum histidine after an oral load is a characteristic of the metabolic defect. Control E. gave only a small response.

*Histidine in cerebrospinal fluid.* Only in patient C. G. histidine could be estimated in cerebrospinal fluid. On two different days (9/2 1965 and 14/6 1965) we found a tenfold elevated value: 2.20 and 2.02 mg/100 ml. (serum histidine 17.0 mg/100 ml on 14/6 1965). According to Perry & Jones [17] the histidine concentration of normal cerebrospinal fluid is 0.20 mg/100 ml.

*Excretion of histidine.* The Tables 1 and 2 contain data about the excretion of histidine per gram of creatinine, estimated in the 24-hr urine, as far as a complete collection could be accomplished. (Estimation of precise 24-hr excretions was given up, because it is seldom possible to get sufficiently reliable collections.) C. G. was compared with a control baby E. of nearly the same age and weight and with a comparable creatinine excretion of about 75 mg/24 hr. She excreted about 4 times as much histidine as her control. There was no close relation between intake and excretion of histidine.

TABLE 2. *Histidine in serum and urine of the older patients M. t. B., Y. B. and control H. (boy 28/7-55, 3.3 kg, creatinine excretion of the same magnitude)*

| | Date of analyses | Histidine intake (mg/kg/24 hr) | Fasting serum histidine (mg/100 ml) | Histidine in urine (mg/g creatinine) |
|---|---|---|---|---|
| Patient M. t. B. | 13/7–64 | (83)* | 6.1 | 493 |
| | 9/8–65 | (53)* | 10.3 | 590 |
| Patient Y. B. | 15/11–66 | (40)* | 7.4 | 771 |
| Control: H. | 15/2–66 | (40)* | 1.74 | 150 |

On free diet; histidine intake roughly calculated.

TABLE 3. *Normal children on a free diet histidine in 24 hour urine samples*

| Case sex, age in years at examination | Histidine (mg/g creatinine) |
|---|---|
| 1. A. B. ♀, 4/12 | 118 |
| 2. P. T. ♂, 8/12 | 128 |
| 3. E. d. M. ♂, 7/12 | 256 |
| 4. C. d. M. ♀, 9/12 | 165 |
| 5. H. T. ♂, 7/12 | 202 |
| 6. Ch. d. M. ♂, 7/12 | 145 |
| 7. L. T. ♀, 8/12 | 283 |
| 8. H. T. ♂, 10/12 | 300 |

TABLE 4. *Six cystinuric children on an average diet. Excretion of histidine, mg/g creatinine*

| Case sex, age in years at examination | Histidine (mg/g creatinine) |
|---|---|
| 1. H. P. ♂, /12 | 467 |
| 2. G. P. ♀, 1/12 | 678 |
| G. v. P. ♀, 1/12 | 660 |
| 3. P. Je. ♂, 2/12 | 330 |
| 4. A. v. P. ♀, 2/12 | 331 |
| 5. P. Je. ♂, 6/12 | 172 |
| 6. K. E. ♂, /12 | 222 |

The two older patients excreted 2 to 5 times as much histidine as their control H. of the same age and weight. However if excretion data are compared with those of 8 normal children on a free diet (Table 3) and with 6 cystinuric children on a free diet (Table 4) they cannot be classified as beyond the normal. Also among the data of Hunter [16] for the excretions of histidine by normal children are a few that reach the level of M. t. B. and Y. B. Adults' values are lower: 20 men from 56–267, 1 women from 58–206 mg/g creatinine. Hunter mentions an incomplete tubular reabsorption of histidine as a possible cause of a high individual histidine output. He suggests an unusual degree of renal immaturity in these children.

*Excretion of histidine metabolites.* In patients with histidase deficiency histidine is metabolised via secondary routes. Now transamination is an important route for degradation. Imidazolepyruvic acid (IPA) is a main metabolite. La Du *et al.* [9] found in their case 1 an output of 16.. mg IPA and 3?8 mg histidine (average values). The patient of Auerbach *et al.* [2] excreted 63 mg IPA and 500 mg histidine. After an oral load of histidine the IPA excretions strongly increased, then patients always show a positive ferrichloride reaction. This is in sharp contrast to normals after a loading dose. IPA is not a convenient parameter for analysis. It can disappear

TABLE 5. *Excretion of histidine metabolites (mg/g creatinine) in young patient C. G. and control baby E.*

| | Imidazole lactic acid M186 (mg/g creatinine) | Imidazole acetic acid M178 (mg/g creatinine) | N-acet. histidine M197 (mg/g creatinine) |
|---|---|---|---|
| Patient C. G. | | | |
| Urine 27/10–64 | 630 | 253 | 77 |
| Urine 20/1–65 | 570 | 279 | 98 |
| Urine 14/6–65 | 274 | —1 | 60 |
| Control F. | | | |
| Urine 31/3–66 | 30 | 31 | 4— |

TABLE 6 *Excretion of histidine metabolites (mg/g creatinine) in older patients M. t. B and Y. B. and control H*

| | Imidazole lactic acid M156 (mg/g creatinine) | Imidazole acetic acid M126 (mg/g creatinine) | N-acetylhistidine M197 (mg/g creatinine) |
|---|---|---|---|
| Patient M. t. B. | | | |
| Urine 12/7–64 | 56 | 10.0 | 6.6 |
| Urine 9/5–65 | 128 | 58 | 11 |
| Patient Y. B. | | | |
| Urine 6/10–65 | 132 | 20 | 26 |
| Urine 15/11–65 | 87 | 21 | 23 |
| Control H. | | | |
| Urine 15/*–65 | 12.9 | 1.6 | 2.7 |

quickly from the urine by chemical conversion and it is lost for the greater part in paperchromatographic procedures commonly used for diagnostic purposes. The other main metabolite, the stable imidazolelactic acid does not bring along such inconveniences. A third metabolite is imidazoleacetic acid, which also can originate from *in vitro* decomposition of IPA. Acetylation of histidine is a minor pathway $\alpha$ N-acetylhistidine has been identified by Shaw *et al.* [*0]. We found in the urines of our patients as well as in normal urines a substance with the same paperchromatographic characteristics as N-acetylhistidine. This substance isolated from a patient's urine by preparative paperchromatography yielded histidine after hydrolysis.

In Tables 5 and 6 the excretions of imidazolelactic acid, imidazoleacetic acid and acetylhistidine of patients and controls are given. In all patients imidazolelactic acid is the compound, excreted in the largest amounts.

The high excretion of these compounds can easily be read from a 2-dimensional chromatogram. In Figs. *b* and *a* such



Fig. _. Urinary imidazoles in control ( ) and in patient C. G. (*b*) (urine 14/*). N tice increased output of imidazolelactic acid (*ILA*), imidazoleacetic acid (*IAA*), N-acetylhistidine (*NAH*) and histidine (*H*). A close-up of / of the chromatograms is reproduced

an imidazole chromatogram" of patient C. G is compared with that of a normal person. The difference is quite obvious.

Thus whenever from a routine screening of urinary amino acids a high excretion of histidine results, the chromatogram of the imidazoles can provide further information whether a histidase deficiency is present or not.

## Comment

The three patients conform to the description in the literature of the children with a histidase deficiency (with the exception of those reported by Woody *et al* [-5], who were found to have a partial histidase deficiency) Our patients also have strongly elevated serum histidine levels; the excretion of histidine per gram of creatinine is high or increased, we found abnormal amounts of imidazolelactic acid, imidazoleacetic acid and α N-acetylhistidine in their urine We suppose that a histidase deficiency of liver and skin both exists as has been proved by several authors [8 14, 26].

When histidinemia is compared with phenylketonuria it can be noticed that the increase of serum histidine in the former disease is less than the elevation of phenylalanine in the latter A diminished tubular reabsorption of histidine in young children [4, 6] and a lower intake of this amino acid contribute to this difference The milder character of histidinemia as compared with phenylketonuria may be connected with the difference in amino acid concentration.

Nevertheless, histidinemia remains a serious disease. From the 18 patients (15 from the literature and 3 own patients) 3 children have a normal intelligence, of the other children 4 are slightly 11 moderately to severely mentally retarded. Two patients are under 2 years of age From the remaining 16 children 14 have speech defects. The high frequency of mental retardation and speech difficulties demand treatment of this metabolic disorder In this respect we cannot agree with the opinion of Hass [15] who states in his description of histidinemia that no treatment is indicated. Probably the results of the treatment will be age dependent the cerebral damage will be less in the youngest children. About a possible recovery of the cerebral damage nothing is known but at older age it cannot be expected. For that reason we treated the youngest patient with a diet restricted in histidine

## Summary

Three cases of histidinemia are described. All patients were mentally retarded.

Quantitative data obtained from columnchromatographic and paperchromatographi methods are given. These data concern the concentration of histidine in serum and cerebrospinal fluid, the urinary excretion of histidine imidazolelactic acid, imidazoleacetic acid and N-acetylhistidine both in patients and controls.

In histidinemia a high frequency of mental retardation i found It is pointed out that this anomaly must be lassified as a serious disease which lik phenylketonuri should be treated with a special diet early in infancy

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# Treatment of a Patient with Histidinemia

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Out of the 18 patients mentioned in a preceding article [4] 15 have a mild or even severe grade of mental retardation and 14 out of 16 patients above _ years of age exhibited disturbed speech. Analogous to the situation in phenylketonuria in histidinemia the mental retardation may be related with the abnormal histidine metabolism. It may be hoped that normalization of the histidine blood level will prevent cerebral deterioration.

A girl, C. G patient [4], no. 3 of the preceding report was treated with his-tidine-restricted diet. This diet was started at the age of 1 months.

## Preparation of Diet Restricted in Histidine

A protein-restricted diet, containing enough protein to allow a sufficient growth, always contains too much histidine for normalization of the blood level. Preparation of a histidine-restricted diet on basis of natural proteins is not possible. For long-term treatment the pplication of pure l-amino acids is expensive Histidine-restricted food on a base of a casein hydrolysate with some supplements seemed to us practical approach. This basal histidine-poor hydrolysate was kindly manufactured for our patient. The removal of histidine from the hydrolysate by an ion-exchanger however also resulted in complet loss of lysine arginine and tryptophane. Therefore these amino acids were replaced.

During dietary treatment of other inborn errors of metabolism (phenylketonuria) it became clear that it is almost impossible to get a sufficient weight and height increase on a pure synthetic diet [2]. W added some milk, potatoes and vegetables.

It is well known [3] that more calories are required when intact proteins are replaced by free amino acids or a protein hydrolysate. This must be born in mind when preparing synthetic diet. W gave pproximately 800 calories/24 h. (corresponding to 118 cal/kg) which included bout 250 calories from carbohydrates.

Another danger of such a diet is the development of deficiencies. To be considered are:

1 Vitamin deficiencies, especially of the B-group.
2. Shortage of the minerals Ca, Mg and P as well as Fe In addition trace elements as Cu, Zn, Mn, Co, M and I.
3. A deficiency of essential fatty acids as linoleic and arachidonic acids.

In Table 1 the amounts of the different nutrients in the diet can be read. T bles 2 and 3 refer t the amounts of vitamins, minerals and trace lements. Addition of maize oil guarantees a sufficient supply of essential fatty acids.

In Table 4 details concerning the intake of the calculated and administered amounts of essential amino acids are given. Columns 1 and contain data as recommended by Holt & Snyderman [1]. In columns 3 and 4 the amounts of the essential amino acids

N V Nutricia Zoetermeer Holland.

TABLE 1 *Histidine restricted diet*

| | Calories | Protein, g | Fat g | Carbo-hydrate g | Histidine, mg | Lysine, mg | Arginine, mg | Trypto-phane, mg | Threonine mg | Phenyl alanine, mg |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 g histidine-restricted hydrolysate | 40 | 10 | | | 0 06 | 0.71 | 3.6 | 0 | 420 | 456 |
| 130 ml cow' milk | 84 | 3.3 | 3 | 4 8 | 82 | 10 | 110 | 44 | 140 | 160 |
| 40 ml maize oil | 360 | | 40 | | | | | | | |
| 40 g glucose | 100 | | | 40 | | | | | | |
| 30 g dextrine-maltose | 120 | | | 30 | | | | | | |
| 50 g spinach (boiled) | 8.5 | 1.3 | 0.4 | 1 1 | 23.5 | 79 | 55 | 18 | 48 | 47 |
| 50 g pot toes (boiled) | 41 | 1 | | 10 | 17 | 50 | 50 | 14 | 40 | 43 |
| 50 g orange juice | 21 | 0.23 | | 5 | | | | | | |
| 700 ml water | | | | | | | | | | |
| Added as l-amino acid | | | | | | 1000 | 600 | 500 | | |
| | 803 | 15.7 | 43 | 91 | 123.6 | 1360 7 | 718.6 | 576 | 648 | 606 |

(in 12 g of hydrolysate with supplements) and total intake respectively re tabulated.

## Course of the Treatment

At first the histidine intake was 122 mg, corresponding to 19.5 mg/kg/day This was 53 % of the required intake for normal children as recommended by Holt & Snijderman [1] Thirteen days after treatment was started, fasting serum histidine was reduced from 17 0 to 2.2 mg/100 ml (Table 5) Despite a slight elevation of the histidine intake the concentration decreased further to 0 77 mg/100 ml.

We felt it would be safer to maintain a serum concentration on a slightly elevated level. Eight days after the addition of 50 ml of milk to the diet (41 mg of histidine) the serum histidine level was 1.05 mg/100 ml As can be seen from Table 5 the excretion of histidine runs parallel to *the intake By lowering the intake* a normal excretion of imidazolelactic acid imidazoleacetic acid and of N acetyl histidine is reached. The analytical methods used are the same as given in the preceding paper [4]

During the following months and after hospital discharge the intake of histidine was fixed at approximately 28 mg/kg/24h, despite a constant slow rise of the serum histidine (Table 6) Haemoglobin wa low and remained so there seemed to be no correlation with serum histidine Serum protein fluctuated on a subnormal level. Growth was definitely inadequate (below the third percentile) No beneficial effect was seen from treatment as can be read from Table 7

When serum histidine rose to the level of 5 mg/100 ml the intake was reduced to 14 0 mg/kg/24 hr Instead of milk, Almiron

TABLE Daily vitamin supply

*2 coated tablets Dumovitamin B complex (Organon)*

| | | | |
|---|---|---|---|
| Vitamin B1 | 3 mg | Vitamin B12 | 6 gamma |
| Vitamin B2 | 4 mg | Folic acid | 1 mg |
| Vitamin B6 | 4 mg | Vitamin A | 2000 I. U |
| Nicotinamide | 40 mg | Vitamin D | 1000 I. U |
| Ca-pantothenate | 20 mg | | |
| Dried medical yeast | 200 mg | | |

TABLE 3 *Daily supply of minerals and trace elements (based on W nitz et al 1965)*

| | | | |
|---|---|---|---|
| MgO | 0.078 g | KJ | 0.030 mg |
| $Ca(H_2PO_4)_2\ 2H_2O$ | 2.230 g | $MnCl_2\ 4H_2O$ | 2.036 mg |
| Pulverised eggshells | 0.730 g | $Zn(Ac)_2\ 2H_2O$ | 0 404 mg |
| | | $Cu(Ac)_2\ H_2O$ | 0.300 mg |
| Ferronicum Sandoz 13 ml 40 mg F | | $Co(Ac)_2\ 4H_2O$ | 0.334 mg |
| | | $(NH_4)_6Mo_7O_{24}\ 4H_2O$ | 0 064 mg |
| | | $Na_2$ glycerophosphate $5H_2O$ | 1.834 g |

TABLE 4. *Requirement and intake of amino acids*

| | Requirement of amino acids | | | |
|---|---|---|---|---|
| | (1) mg/kg of bodyweight | (2) For child of 7 kg, mg | (3) Content of 12 g of hydrolysate + supplement, mg | (4) Total intake mg |
| Histidine | 34 | 233 | 0 96 | 123 6 |
| Isoleucine | 119 | 833 | 1183 | 1463 |
| Leucine | 150 | 1050 | 1068 | 2108 |
| Lysine | 103 | 721 | 0.72 + 1800 | 1801 |
| Methionine | 45 | 315 | 450 | 434 |
| Phenylalanine | 90 | 630 | 450 | 904 |
| Threonine | 87 | 609 | 420 | 643 |
| Tryptophane | | 154 | 0 408 | 576 |
| Valine | 103 | 778 | 1044 | 1370 |

TABLE 5 *Normalization of fasting serum histidine and of the excretion of histidine and metabolites after administration of a diet restricted in histidine*

| Diet | | Histidine restricted diet-start on 14/6 | | | | |
|---|---|---|---|---|---|---|
| Date of analysis | 14/6 | 1 /6 | 21/6 | 27/6 | 4/7 | 1_/7 |
| Intake of histidine mg/kg/24 hr | 105 | 19 7 | 19 7 | 19 8 | 22.2 | 78.4 |
| Serum histidine mg/100 ml | 11 9 | 10 1 | 8.6 | 2 | 0 7 | 1.85 |
| Histidine in urine mg/g creatinine | 1603 | 808 | 603 | 182 | 82 | 122 |
| Imidazolelactic acid in urine mg/g creatinine | 174 | 183 | 93 | 13 | 2.0 | 10 |
| Imidazoleacetic acid in urine mg/g creatinine | 3 | 87 | 49 | _8 | 1.0 | 2_ |
| N-acetylhistidine in urine mg/g creatinine | 69 | 28 | 28 | 7.2 | 2.2 | — |

Table 6 *Course of treatment with a histidine restricted diet*

| Dat | 27/7 | 9/8 | 23/8 | 7/9 | 23/9 | 19/10 | 11/11 | 8/1 | 10/1 | 12/1 | 31/3[b] | 4/5[b] | 19/7[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Histidine intake mg/kg/24 hr | 27.9 | 26.9 | 26.2 | 27.0 | 25.4 | 25.8 | 23.9 | 23.[illegible] | 23 | 22.8 | 11.9 | 11.6 | 14.9 |
| Serum histidine, mg/100 ml | 1.41 | 1.56 | 1.[illegible]1 | 2.31 | 2.73 | 3.31 | 3.39 | 4.08 | 5.27 | 4.35 | 1.02 | 0.82 | [illegible].50 |
| Dat | 29/7 | | | 6/9 | | 18/10 | 8/11 | | | | | | |
| Haemoglobin, g/100 ml | 9.4 | 8.6 | 9.2 | 10.7 | 9.3 | 10.4 | 9.5 | — | 10.9 | — | — | 10.2 | 9.2 |
| Total serum protein, g/100 ml | 5.6 | 5.8 | 5.5 | 5.8 | — | 5.7 | 5.4 | — | 6.0 | — | — | 6.2 | 6.5 |

Discharge from hospital on 11/11.
[b] Change of histidine intake to 11.9 mg/kg/24 hr by a mistake of the mother
Readjusted on 14.9 mg/kg/24 hr

Table 7 *Growth of patient*

Start of histidine restricted diet at 14/8–65.

| Dat | Age in months | Weight, g | Length, cm |
|---|---|---|---|
| Dec. 1964 | 6 | 5540 | 62.5 |
| March 1965 | 9 | 6020 | 64.5 |
| April 1965 | 12 | 6690 | 68 |
| Sept. 1965 | 15 | 7300 | 70 |
| Jan. 1966 | 19 | 8200 | 72 |
| July 1966 | 25 | 9000 | 77.5 |
| Jan. 1967 | 31 | 10,200 | 81 |

A (N.V. Nutricia, Zoetermeer, Holland) containing 1.8 % protein was used. Unfortunately only half the prescribed Almiron A was given at home, which resulted in a daily histidine intake of only 11.9 mg/kg/24 hr. Serum histidine fell drastically. After re-establishment of the intake at 14.9, a satisfactory concentration of 2.50 mg/100 ml was reached again. Treatment is being continued.

## Comment

At this moment little can be said about the patient's mental condition. Undoubtedly her mental development is retarded, as can be concluded from several psychological examinations. However, the hospitalization of the child from the 4th day until the 17th month of life may have been partially responsible for the retardation. On the other hand motor function development and speech behaviour show a favourable progress during the period of observation. A detailed description of the child's mental development will be given in the near future.

## Summary

In a young patient with histidinemia, treatment with a diet restricted in histidine was started. A complete normalization of the serum histidine concentration could be obtained. The excretion of histidine, imidazolelactic acid, imidazoleacetic acid and N-acetylhistidine decreased to a normal level. Details about the composition of the diet and a brief description of the course of the treatment are given.

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# Respiratory Failure in the Newborn

## *The Techniques and Results of Intermittent Positive Pressure Ventilation*

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Respiratory failure is a common cause of death in the neonate where the Respiratory Distress Syndrome (RDS) atelectasis and aspiration are conditions with a high mortality In the terminal stages hypoxaemia, hypercapnia together with acidosis are the main features of the biochemical upset. Despite the frequency of respiratory failure artificial ventilation has not been used very widely Most reports include only a few cases [2, 4 6 7 8 11 14, 19 20 23 26 29]. The great technical difficulties [1 10 17 18 19 24 25 28, 29] involved in maintaining prolonged ventilation of a small newborn infant combined with its low resistance to infections and the complexity of the underlying pulmonary and circulatory disturbances, are responsible for the hesitancy of embarking on such a program During the course of the last decade it has been demonstrated that this form of treatment was successful in many infants, despite the extreme conditions that were often present before treatment was commenced. This was necessary since the treatment itself has many inherent risks. It is the purpose of this paper to present our techniques, since we feel that we have overcome some of the problems and to discuss some of the clinical details in the management of these infants.

### Techniques

#### *The indications for artificial ventilation*

No definite criteria were used in the selection of infants for artificial ventilation. Initially only those infants that were clinically moribund and in gross respiratory and circulatory collapse were subjected to this treatment. Later in the series an important sign proved to be a slowly increasing hypercapnia, after a period of normal or even low carbon dioxide tension ($Pa_{CO_2}$) early in the disease. This usually followed after a progressive fall in arterial oxygen tension ($Pa_{O_2}$) This, accompanied by clinical signs of exhaustion and progressive deterioration was considered the stage at which artificial ventilation should be commenced. The clinical signs of progressive deterioration were noted from an observation chart which showed a record of quarterly to halfhourly observation of respiration rate Silverman score oxygen concentration, heart rate and clinical observation of the infant.

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*Intubation and fixation of tubings*

Prior to intubation gastric and naso-pharyngeal aspiration was performed and the infant given 100 per cent oxygen by mask. Naso-tracheal intubation was used in all the infants with Portex tubes (2.5–3.5 mm) using an Oxford Infant Laryngoscope. The naso-tracheal tube was only changed when suction failed to overcome an obstruction.

The infant was nursed in a specially constructed crib [1] made for the purpose which was placed inside an incubator to facilitate environmental temperature and humidity control. The head of the infant was supported firmly inside the crib using soft sponge and respirator tubings after entering the incubator were fixed to the brackets on the head end of the crib (5).

*The respirator*

A Bird mark VIII with a flow cartridge to control the inspired oxygen concentration from 100 per cent oxygen to atmospheric air was used. Since the flow cartridge was only a late addition to the respirator grading of the oxygen concentration had not been possible in the earlier part of the series. A T-connection leading to a Beckman oxygen analyser was incorporated into the circuit. The adequacy of ventilation of the infant was judged clinically and by blood gas determination. The pressures applied ranged between 40 and 18 cms of water and a negative pressure of minus 2 or 3 cms $H_2O$ often used in the expiratory phase to overcome the resistance in the narrow naso-tracheal tube. The respirator circuit was cleaned by placing it in a solution of Rodalon ® (Benzalkonium chloridum NFN) with concentration of 1 g per 10 liters of water for a period of 15 minutes and thereafter in fresh running water for 30 minutes. Bacteriological cultures were taken from the respiratory circuit before use.

*Humidification*

In order to obtain satisfactory humidification during naso-tracheal intubation a thermostatically controlled water bath was placed in the circuit (15). A thermocouple was introduced into the tubing so that its tip was at a point immediately before the tubings entered the incubator. The thermostat was adjusted so as to obtain full saturation of the inspired gases prior to entering the incubator which was noted by ensuring condensation up to that point. The temperature at the thermocouple was kept 1–1.5°C less than the temperature inside the incubator by adjusting the thermostat. By controlling the temperature in this way the infant was not overheated nor did condensation take place in the tubings within the incubator. The deep rectal temperature of the infant and the temperature of the thermocouple tip enabled us to know that the humidification in the tracheo-bronchial tree was about 75–80 per cent in all infants where this humidification system was used. In this way drying out of the secretions and the danger of blocking of the naso-tracheal tube was kept to a minimum so the same tube could often be used for 2–3 days.

*The clinical management of the infant*

Repeated clinical assessment by a physician was carried out and the length of time between two clinical assessments were never greater than six hours. Specially trained medical students were assigned to the infant. The numerous clinical and technical details required frequent and meticulous observation. The temperatures of the room, incubator, humidifier, thermocouple and rectum were recorded. The inspiratory pressure and expiratory negative pressure, flow rate, sensitivity and oxygen concentration of the respirator were likewise recorded. Recordings of respiration rate, heart rate, intake, output and the time and yield of suctioning were made. The position of the infant for postural drainage and physiotherapy were also noted. The observation scheme included a column for medications, investigation and remarks.

[1] Obtainable from Dameca, Islevdalvej 111 Brønshøj, Denmark.

*Feeding*

In the majority of cases the infants received 65–100 ml/kg/24 hours of 5 or 10 per cent glucose or a glucose Darrow solution (3:1) intravenously. As soon as bowel sounds were heard, and in some cases before, gastric feeding was attempted using a gastric tube. The tube was removed between feeds to avoid the accumulation of secretions. Careful assessment of retention was carried out before repeat feeds were given. It was our aim to provide half milk-5 % glucose water feeds by this route as early as possible. In order to facilitate the passage of meconium, small normal saline enemas were often given early in the disease.

*The free airway*

A well ventilated infant was usually a contented one settling down and sleeping shortly after respirator treatment was commenced. The restless infant therefore required careful and immediate evaluation. By observation of these infants we found the differential diagnosis of a restless infant to be as follows:

1. Blocked naso-tracheal tube.
2. Inadequate ventilation.
3. Tracheo-bronchial secretions.
4. Naso-pharyngeal secretions.
5. Hunger.
6. Distended stomach.
7. Full bladder.
8. Full rectum.

With a knowledge of the above it was possible to approach the restless infant in a practical way. Auscultation of the chest and of the disconnected nasotracheal tube revealed the immediate danger of blockage or secretions. Rhonchi over the chest fields without being heard over the disconnected naso-tracheal tube usually cleared after adequate suctioning of the nasopharynx. The most common causes of a restless infant were full bladder, naso-pharyngeal secretions or hunger. The complication of pneumothorax was always kept in mind.

*Postural drainage and physiotherapy*

The practical problems encountered in the movement of the infant in the early part of the series resulted in little effective postural drainage. With the development of the crib the infant and the tubings could be moved as one unit and halfhourly postural drainage and physiotherapy were commenced. The infant was nursed in the left or right half lateral positions, the supine position was avoided whenever possible. Between the two lateral positions the infant was placed in the Trendelenburg or anti-Trendelenburg position when indicated. Auscultation of the lung fields was carried out frequently during this program. A method of physiotherapy using soft percussion over the anterior and posterior lung fields was used.

*Suctioning of the infant*

Suctioning of the naso-pharynx was performed regularly before the accumulation of secretions became evident. When ileus was a feature of the disease the gastric contents was likewise aspirated regularly. Tracheobronchial suction was performed only when indicated using sterile gloves and sterile atraumatic tipped side-holed, size 5 French feeding tubes. Adequate oxygenation before and after this short procedure was carried out. Secretions were sent off daily for bacteriological study.

*Biochemical assessment*

The determination of the arterial blood gases and the pH was performed every 12 hours unless indicated earlier. The arterial blood taken and measured by one of us (R. C.) in the anaesthesiology laboratory was obtained from an indwelling umbilical artery catheter or from repeated arterial punctures of the radial, femoral or temporal arteries. The samples (0.3–1.0 ml) were immediately placed on ice and the analyses were performed in most instances within ten minutes, and in all cases in less than half an hour following sampling. The arterial blood was taken after supplying 100 per

cent oxygen by mask for 10 minutes at 10 liters per minute as recommended [2]. Oxygen tension was measured with Clark type electrode (Radiometer type E 5046), carbon dioxide tension with a Radiometer type E 5036 electrode and pH was measured with a Micro pH electrode (Radiometer type E 50 1). Corrections for temperature differences between electrode (38°C) and infant were performed according to Severinghaus [22] for oxygen tensions up to 200 mm Hg and according to Hedley-Whyte et al. [10] for oxygen tensions above this value.

In the earlier part of this series and in those cases where arterial sampling proved difficult, $Pa_{CO_2}$, pH and Base Excess were measured by arterialised capillary blood obtained from the heel. Temperature correction t 38°C was carried out, this being the average rectal temperature in 50 consecutive arterial punctures performed in newborn infants with respiratory distress. The arterialised capillary blood was measured by the hospital central laboratory. The validity of the temperature corrections is based on the assumption that the factors used also apply to neonatal blood. This may not be the case at oxygen tensions below about 200 mm Hg due to the presence of fetal haemoglobin in neonatal blood. The error introduced by this assumption is however considered t be less than the error introduced by making no temperature corrections. Temperature differences of up t 5°C were noted.

*Radiological assessment*

An x-ray of the thorax was obtained if possible *immediately prior to* intubation and within few hours after intubation. Thereafter daily x-rays were taken unless otherwise indicated.

*Electrical monitoring*

Electrocardiographic monitoring using small chest electrodes was used when indicated. A rectal temperature probe was used constantly. It as our aim to keep the interior of the incubator as simple and as practical as possible. This allowed easy access to the infant at all times.

*Weaning the infant from the respirator*

A definite criteria was set out during the course of this series. The clinical, biochemical and radiological evaluation of the infant was considered in the individual infant. An improvement in the general clinical condition of the infant together with satisfactory ventilation of both lung fields confirmed by the blood gas analyses and radiological examination were strong indications for removing the infant from the respirator. An attempt was made to wean the infant off the respirator at the earliest possible moment because of the risks involved in prolonged artificial ventilation.

*The method of weaning the infant from the respirator*

The infant was connected for electrocardiographic monitoring. The respirator tubes were disconnected from the nasotracheal tube which was left in position. The incubator contained the maximum oxygen concentration of 0 per cent and the humidity within the incubator was in most instances as great as 85–90 per cent. A funnel supplying 100 per cent oxygen with a flow rat of 10 liters per minute with full humidification as placed over the nasotracheal tube. Minute by minute recorded clinical evaluation of the lung fields together with respiration and heart rat were made. It was attempted t keep the infant off the respirator for at least 10 minutes in the first instance. Thereafter longer times were attempted similar fashion. Blood gas analyses and radiological examination were performed either during the course of weaning or shortly after stable state off the respirator had been achieved.

*Extubation*

Once the infant showed a satisfactory condition off the respirator for period of 30 60 min extubation was considered.

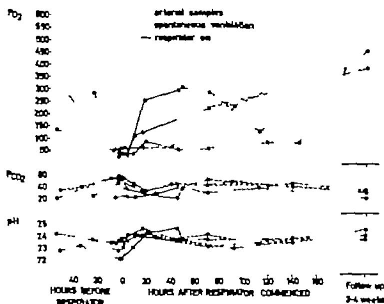



Fig. 1 Course of $Pa_{CO_2}$ (mm Hg), $Pa_{O_2}$ (mm Hg) and pH in four survivals in which arterial samples were measured. Inspired oxygen concentration was 100 % in all instances.

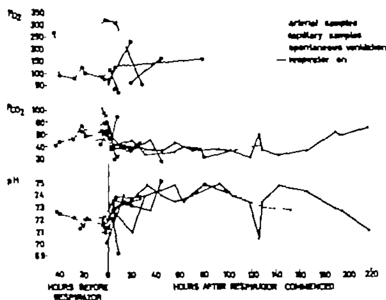



Fig. — Course of $Pa_{O_2}$ (mm Hg), $Pa_{CO_2}$ (mm Hg) and pH in nine deaths. Inspired oxygen concentration was 100 % in all instances. Capillary samples were obtained after arterialisation.

Table 1. *Summary of the material*

| Data | Deaths | Survivals |
|---|---|---|
| Total number of infants | 9 | 5 |
| Mean weight (g) | 1688 | 2810 |
| range | (950–2350) | (2400–3600) |
| Mean gestational age (weeks) | 32 | 35 |
| range | (26–37) | (34–37) |
| Sex (M/F) | [illegible] | [illegible] |
| Complications during pregnancy and delivery | 7 | 3 |
| Clinical asphyxia at birth | 7 | 4 |
| RDS without additional complications | 2 | 2 |
| RDS with additional complications | 7 | 3 |

However there was a great individual variation according to the assessment of the infant. Immediately prior to extubation the trachea was suctioned followed by a period of oxygenation. The gastric and nasopharyngeal contents were then aspirated and extubation was carried out. The infant was now manually ventilated with 100 per cent oxygen for a short time. Intensive minute by minute assessment of the infant was carried out as described above as this procedure was considered a critical phase in the treatment. Reintubation with the continuation of respirator treatment was sometimes necessary

*The further management of the infant*

Humidified oxygen in concentrations high enough to maintain an arterial oxygen tension of 100–150 mm Hg [12] was supplied by funnel at six to ten liters per minute. The oxygen concentration was gradually reduced over a period of days according to the arterial oxygen tension. If the arterial oxygen tension was not available the oxygen was reduced according to the clinical response of the infant

Postural drainage, physiotherapy and frequent suctioning of the nasopharynx were continued. Repeated bacteriological examination of naso-pharyngeal secretions was performed to detect any change in the type or sensitivity of the invading bacteria.

Table 2. *Clinical course of survivals and deaths before, during and after respirator treatment*

| | Deaths | Survivals |
|---|---|---|
| Haemolytic disease of newborn requiring immediate exchange transfusion | 3 | 1 |
| Cardiac failure | 5 | 3 |
| Cardiac arrest | 1 | 0 |
| Early aspiration | 1 | 2 |
| Apnoea | 2 | 0 |
| Convulsions | 2 | 0 |
| Collapsed state at intubation | 0 | 3 |
| *Mean age at intubation (hr)* | 18 | 51 |
| Range | 0–52 | 27–72 |
| *Complications arising during respirator treatment* | | |
| Intubation right main bronchus | 1 | 1 |
| Accidental extubation | 3 | 0 |
| Pneumonia | 1 | 2 |
| Blocked tube requiring extubation | 2 | 1 |
| Bronchospasm | 1 | 2 |
| Tension pneumothorax and haemothorax | 1 | 0 |
| Cardiac failure | 4 | 0 |
| Convulsions | 3 | 0 |
| *Mean duration of respirator treatment (hr)* | 51 | 29 |
| Range | 10–200 | 12–47 |
| *Complications on weaning infants from respirator* | | |
| Cardiac arrest | 2 | 0 |
| Apnoea | 1 | 0 |
| Atelectasis | 0 | 1 |
| Mild laryngeal stridor | 0 | 1 |
| *Complications following successful weaning from respirator* | | |
| Pneumonia | 1 | 4 |
| Cardiac failure | 1 | 1 |

## Material

Fourteen consecutive infants are included in this series treated between April and December 1966. The clinical and biochemical data are to be found in Tables 1 and 2 and Figs. 1 and

## Results

The clinical and biochemical course of the infants are in Table 2 and Figs 1 and

TABLE 3 *Necropsy findings*

| | |
|---|---|
| Laryngeal ulceration | 1 |
| Hyaline membrane | 8 |
| Pneumonia | 4 |
| Immature lungs | 1 |
| Subarachnoid haemorrhage | 3 |
| Brain cell degeneration | 8 |
| Positive bacteriological cultures from organs | |

2. Five infants survived and necropsies were performed on all nine infants that died. The necropsy results are in Table 3

## Discussion

### *Indications*

In previous reports where artificial ventilation has been used in the treatment of pulmonary insufficiency of the newborn the conditions before the commencement of respirator treatment have been extreme In a series of twenty infants reported by Delivoria Papadopoulos *et al* [7] clinical signs of respiratory arrest gasps of less than 3 per minute cardiac arrest or a rate of less than sixty per minute and central and peripheral cyanosis in 100 per cent oxygen were present These clinical signs together with a pH of less than 6.8 $Pa_{CO_2}$ greater than 120 mm Hg and $Pa_{O_2}$ in 100 per cent oxygen of less than 30 mm Hg were found when these infants were first intubated and ventilated. Such extreme disturbances could not be expected to yield many positive results and this was to be our experience in the early phase of this small series.

Conventional conservative therapy providing increased oxygen concentration glucose bicarbonate solutions for the correction of the metabolic acidosis and antibiotics was used. This therapy did not prevent these infants from subsequent deterioration in respiratory and cardiac failure

The failure of oxygen in high concentration to maintain a satisfactory arterial oxygen tension is explained by increasing right to left shunts, either intrapulmonary by circulation through poorly ventilated areas or through patent foetal channels [27 30].

Diminished surfactant activity and increasing atelectasis are considered to play an important role in the progressively falling arterial oxygen tension [9]. In RDS a falling arterial oxygen tension appears to be the initial feature of the blood gas measurement [26] (Fig. *). At this stage the acidaemia is probably more an expression of uncorrected birth asphyxia. The prolonged period of progressively decreasing arterial oxygen tension with a near normal or low $Pa_{CO_2}$ would strongly suggest that compensation by hyperventilation for increasing atelectasis and acidaemia is taking place The hypercapnia that follows would appear to be at the end stage of compensation when exhaustion and hypoxaemia, with their secondary effects, are manifest. This rise in $Pa_{CO_2}$ is perhaps the earliest indication that compensation is failing. We have used this as our indication for artificial ventilation in the latter part of our series. Infants with associated complications such as cardiac failure and the presence of a low weight are features that appear to hasten this onset of decompensation which may now occur at a higher oxygen tension. These infants therefore require special attention.

Artificial ventilation, in most instances, was able to raise the arterial oxygen ten-

sion and to return the $P_{aCO_2}$ and pH to physiological limits, the latter taking place more rapidly (Figs. 1 and ?) The indication for artificial ventilation is further stressed by the fact that the pathology of RDS i considered to be self limiting [9] if the secondary effects of re spiratory failure can be successfully pre vented or overcome during these first 2 or 3 days of life Prolonged deviation from a physiological stage must allow the complex pathologies to become established and perhaps irreversible Complica tions such as cardiac failure cardiac arrest convulsions and apnoea which occurred during the course of the respiratory disease in our series (Table ) may in this w y be voided. Likewise the high incidence of brain cell degeneration may also be diminished (Table 3) Intensive clinical observation with repeated blood gas analyses are likely to prove the best guide in the individual infant. It should be our aim to commence treatment immediately conservative methods have failed and before progressive respiratory failure with its complications have advanced too far

*Management*

Some workers [1 16 .4] involved in the treatment of tetanus neonatorum use tracheotomy during the course of prolonged artificial ventilation and others [., 7 11 20] treating pulmonary insufficiency secondary to RDS have also fav oured this route

The recent publications [14 19 ?0] on the treatment of RDS atelectasis and spiration have shown nasotracheal in tubation to be an effective and well tole rated procedure The advantages of naso-tracheal intubation over tracheotomy are self-evident and is probably responsible for the increased interest being shown in this field. The use of a smaller tube than otherwise would be tolerated by the trachea, to allow the humidified gases to reach the mucosa of the trachea beneath the naso-tracheal tube, has been stressed by Jackson Rees [18]. In our series laryngeal ulceration was present only in one infant coming to autopsy this infant was the first of our cases before the tech nique was improved and it suffered from gross bronchopneumonia for seven days while on the respirator

Fixation of respirator tubings to the infant together with the possibility of regular postural drainage has proved a difficulty Accidental extubation with subsequent cardiac failure and convulsions despite immediate reintubation and bronchospasm, with respiratory difficulty caused by movement of the naso-tracheal tube in the airway also occurred. The development of our technique for tube fixation has corrected these serious problems.

The incorporation of the oxygen flow cartridges to grade the oxygen concentrations together with the humidification system has made the Bird respirator suitable for our purposes. The efficiency of the ventilation should be evaluated from clinical but more important from blood gas analyses. Rapid corrections of the $P_{aCO_2}$ were obtained in all cases (Figs. 1 and 2) and hyperventilation was also noted. Since the arterial oxygen tension was sometimes seen to increase rapidly after the commencement of respirator treatment it was also necessary for the eval ation of the rterial oxygen

tension so that the appropriate adjustments could be made to the inspired oxygen concentration as an attempt to maintain an arterial $Pa_{O_2}$ around 100–150 mm Hg

Sedatives and neuro-muscular blocking agents which are used in the treatment of tetanus neonatorum with artificial ventilation are not considered necessary in this condition and make assessment of adequate ventilation so much more difficult The approach that we have outlined to the restless infant has been most effective Since the pressure of the respirator that eventually reaches the bronchial tree depends on the resistance of the circuit used it is difficult to compare our pressures with those of other authors. Blocked naso-tracheal tubes requiring extubation have not reoccurred since we have used the present humidification system

Like Thomas *et al.* [29] we have noted on occasions a marked deterioration on suctioning of the naso-tracheal tube Alveolar collapse and increased venous return may be responsible for this [°1]. A short period of hyperinflation has been used and should perhaps be used more often following suctioning. The suction catheter used in this series is not considered satisfactory because of its relatively large size, and a new catheter is being designed. More refined and effective techniques are necessary

The ability to provide adequate calories by an intravenous route remains a problem and in some infants feeding has been performed by gastrostomy [°9]. Ileus, a common feature in infants being artificially ventilated, has been treated by Smythe using 0.25 g of potassium chloride in tetanus neonatorum [23].

It has been shown that there is a poor correlation for $Pa_{O_2}$, $Pa_{CO_2}$ and pH between arterialized capillary blood obtained from the heel and arterial blood during the first 24 hours after birth. For $Pa_{O_2}$ this difference is found throughout the whole neonatal period [13]. Umbilical artery catheterisation or repeated arterial puncture are therefore necessary The radial artery has been used extensively in those cases where umbilical artery catheterisation failed or after the catheter was removed.

The risks involved in artificial ventilation favour an early removal of the infant from the respirator on the other hand treatment should be continued as long as the disease process is considered to be severe usually for the first 3 or 4 days after birth. An increasing $Pa_{O_2}$ and a regression of radiological changes together with a reduction in the pressure needed for adequate artificial ventilation are indications of a regression in the active disease process.

Infection is undoubtedly the most difficult and the most important process to avoid. Early attention to postural drainage and physiotherapy prior to intubation, improved techniques in intubation such as cleaning the nasal passage with a sterile swab [18] and refined techniques of aseptic tracheal suction would be expected to help Likewise the continuation of postural drainage physiotherapy and naso-pharyngeal suction during and after respirator treatment are important. Frequent radiological examination allows early detection of pneumothorax, atelectasis or pneumonia and thereby an early therapeutic program commenced.

## Summary and Conclusions

Respirator treatment in 14 newborn infants with the respiratory distress syndrome is described, there were five survivals. Intensive clinical observations and repeated arterial blood samples are the prerequisites for early detection of respiratory failure Improved techniques of naso-tracheal tube fixation have re duced the inherent risks in the treatment Aseptic techniques, postural drainage and physiotherapy have not been adequate to prevent bronchopulmonary infection.

The early detection of progressive respiratory failure together with improved techniques, particularly those leading to the prevention of infection, should make this treatment effective in the management of the progressive respiratory failure secondary to RDS and other causes, in the neonate

## Acknowledgements

We wish t express our appreciation t Dr I. Tygstrup and Dr L. Molsted Pedersen of the Department of Pathology for their cooperation in this study and to Mrs. Inga Brevadt for technical help. W are also most grateful to the medical students and the nursing staff for their constant vigilance and help.

## Addendum

Since the completion of this study a further 18 infants have been treated on the respirator with 8 survivals. The lowest weight of an infant that survived the treatment being 1120 g

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# Endotracheal Tube Fixation and Postural Drainage in Prolonged Artificial Ventilation of the Newborn

## *Description of a New Apparatus*

*by* R. COOKE[1] B. FRIIS-HANSEN and M LUNDING

Prolonged artificial ventilation in the newborn has been used by a number of workers in the treatment of the respiratory distress syndrome in other cases of pulmonary insufficiency and in tetanus neonatorum [1 3 4 6 8, 9]

Tracheotomy orotracheal and nasotracheal intubation have been used, of these nasotracheal intubation has proved the method of choice [5 7].

A great technical problem has been to fix the endotracheal tube securely to the infant in order to·

1 avoid accidental extubation,
– allow regular postural drainage
3. allow easy access for suctioning and feeding,
4 enable scalp veins to be used for intravenous therapy
5. avoid damage to skin by fixation methods.

The importance of postural drainage during the course of respirator treatment has been stressed [2, 6 9]

Here we describe a simple and effective apparatus that has corrected all the technical problems listed above

### Description of Apparatus

A plastic crib[2] has been designed to accommodate infants in the weight range 1–3 kg (Figs. 1 2, 3)

The main body of the plastic crib is 36.5 cm long, 20 5 cm wide and 6 cm high. This part of the crib has two features

1 A slit 17 5 cm 2.5 cm is placed in the side of the crib for the placement of an x ray plate under the infant.

2. There are holes for fixation of the in fant hands and legs with bands.

The head of the crib is 16 cm long, 16.5 cm wide and 11.5 cm high. Two brackets may be fixed to the head end f the crib for fixation of the respirator tubi gs.

The crib is supported at the head and tail end so that the crib is elevated 5.5 cm off the base. This allows tilting of the crib laterally to 30 degrees.

### Discussion and Practical Applications

The crib has been used duri
f prolonged artificial ve. [2]
of the newborn infant with
insufficiency and was placed

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[2] The crib is obtainable from Dameca, Islevdalvej 211 Rødovre, Denmark.

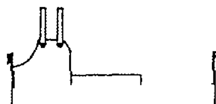


Fig. 1. The crib seen from one side.

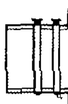


Fig. 3. The crib seen from above, without the support.

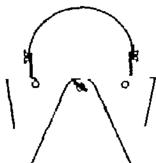


Fig. 2. The crib seen from the tail end.

incubator for environmental temperature and humidity control. Respirator tubings from a modified Bird mark VIII were fixed to the head of the crib after entering the incubator.

The infant was placed on a soft mattress on the floor of the crib with a space left available for an x ray plate to be inserted. The head of the child was fixed with soft sponge to prevent movement (Fig. 4).

Fig. 4. The head of the infant is fixed by soft rubber sponge on either side. Respirat[illegible] tubings entering the incubator are attached to bracket situated on the head end of the crib.

The infants were nursed in the right and left semilateral positions. The fixation of the respirator tubings to the crib and the immobilisation of the infant's head allowed movements of the infant and the tubings without accidental extubation.

The postural drainage that this crib affords should be used together with physiotherapy in an attempt to prevent and treat pulmonary infection. Not only lateral movements are possible with this crib but also the Trendelenburg and the anti-Trendelenburg positions can be used by tilting the bed of the incubator.

The difficulties and hazards in manoeuvring these infants during radiography is minimized by using the slit for positioning the x ray plate. The infant may have to be moved slightly in order to position the infant correctly over the x ray plate.

Since procedures requiring immobilisation of the infant were regularly performed it was necessary to make some simple and effective means of securing the infant. This was done by securing hands and legs to the holes already described. Mobilisation was reestablished as soon as possible.

The crib may be used in the management of any of the respiratory diseases of the neonate and used inside or outside an incubator.

## Summary


A plastic crib has been designed to facilitate fixation of endotracheal tubings, regular postural drainage, easy access for suctioning and feeding, minimal damage to the skin and readily accessible scalp veins during prolonged respirator treatment in the newborn. Since the apparatus has been in use there have been no cases of accidental extubation and minimal problems in manoeuvering the infant.

Further the crib allows easy placement of the x ray plate and fixation of the limbs when necessary. The crib may be adapted easily for those infants being managed with tracheotomy. It should have a wide application in the management of all neonates with pulmonary disease both in the preventative aspects of pulmonary infection with or without respirator treatment and in the management of established infection.


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# Hiatus Hernia in Infancy

*by* BENTE M. SØRENSEN

Just as in adults, hiatus hernia in new born and older infants is found in two forms, sliding hernia and para-oesophageal hernia. In *sliding hernia* the cardiac end of the stomach is displaced upward through a too wide hiatus and the oesophagus shortens longitudinally The slanting direction at which the oesophagus debouches into the stomach straightens out, so that the sphincter effect of the crus diaphragmaticus is insufficient and closure between oesophagus and ventricle is compromised. The symptoms in sliding hernia are due to regurgitation of the stomach contents into the oesophagus.

In *para-oesophageal hernia*, which is less common (about 10 per cent, Thomsen [1 ] the cardia lies in position below the diaphragm, but the fundus or a large part of the stomach is displaced into the thorax The symptoms are not due to regurgitation but to obstruction and although uncommon, incarceration and gangrene of the herniated part of the stomach may develop, with perforation.

Neuhauser & Berenberg [10] in 1947 described 1[illegible] cases of hiatus hernia with regurgitation in infants They stated that the symptoms could be relieved by the infants being kept in the sitting position during and after meals, and in the most severe cases, by letting them sit half up day and night. A follow up several months later showed that the symptoms had disappeared and that regurgitation could no longer be demonstrated by radiological examination.

Astley & Carré [4] carried out this treatment in a large number of cases, and found that the majority became symptom-free, even though a number still showed hernia on radiological examination. Only 3 per cent developed stricture

## Material

Since 1935, a total of 22 infants (12 boys and 10 girls) in the paediatric department of Rigshospitalet and in the Children's Hospital, Fuglebakken, have been treated along the lines laid down by Carré and others. Age at time of hospitalization ranged from two days to 15 months after birth, with mean value of 3 months and 7 days.

In all cases, the symptoms presented were tendency to vomiting, not rarely explosive regurgitation on feeding and in most cases failure to thrive

Nine patients had a low haemoglobin value The anaemia was normochrome in 5 infants and responded to iron. There was occult bleeding in 3 infants, and the other showed haematemesis and melaena on admission

Ten infants were premature one of them also showing periodic pyuria which disap-

peared spontaneously without it being possible to demonstrat any urinogenital malformation. Prior to admission t hospi tal, one patient had undergone operation for pyloric stenosis at the age of 5 weeks, one patient had undergone explorative laparotomy for suspected pyloric stenosis, although no support for this diagnosis could be found on operation, and one patient presented icterus neonatorum which did not require treatment.

In all cases, diagnosis was established by radiological examination of the oesophagus, stomach and duodenum. There was consider able variation in the size of that part of the stomach which constituted the hernia, and there was absolut ly no correlation between the degree of severity of the symptoms and the radiological size of the hiatus hernia. Nor was there any correlation between the intensity of the symptoms and the degree of the regurgit tion demonstrated radiologi cally whether in the Trendelenburg position or not.

In all cases, indications for treatment were vomiting tendency to regurgitation, and failure to thrive

## Treatment and Results

The infant was placed in the sitting position, at first throughout the whole day later for 1– hours after meals. On discharge from hospital all patients with the exception of three were thriving and vomited only rarely The parents were instructed to place the infant in the sitting position after meals for a period of at least *–3 months following complete termina tion of regurgitation or vomiting. In most cases, however it does not appear to have been necessary to continue this regimen for longer than 2 months Thereafter no special precautions with the children have been found necessary

In the case of the 3 patients who did not benefit primarily from the treatment one infant showed roentgenological hiatus hernia together with a 3–4 cm long oesophageal stricture proximal to this which was treated by means of bougie-dilatation with good results, although these were only transient. This infant had not com menced treatment until the age of 8 months, and a stricture had already developed at that stage The other 2 patients had severe oesophagitis complicated by such severe hæmorrhage that resection appeared to be indicated One of these cases terminated fatally on account of pulmonary insufficiency

The remaining 10 infants who became symptom free following primary conserva tive treatment were followed up by clinical and radiological examination during the months of January–February 1966. The period of observation was on the average about 3 years.

Ten patients were found to be completely normal on both clinical and radiological examination. Eight patients were symptom free in spite of a hiatus hernia which could be demonstrated radiologically as well as demonstrable regurgitation in four of the cases One patient complained of an occasional feeling of diffuse oppression in the abdomen with vomiting on a single occasion. There was no failure to thrive in this child, and there had never been any medical attention for any acute condition. Radiological examination showed suspect ed regurgitation but no demonstrable hiatus hernia

## Discussion

Hiatus hernia in infants was mentioned in 10*6 by Åkerlund [1] under the name brachy-oesophagus. In Denmark the condition was first mentioned by Hinde-

Nielsen [8], later by Christiansen [6] and Thomsen [11].

Wamberg [13] in 1947 published 4 cases of so-called congenital brachy-oesophagus, and collected about 70 cases from the lite rature The four children were between the ages of 5–10 years They had all suffered from vomiting from birth or shortly after birth. At that time the disease was described as being a congenitally shortened oesophagus, and various theories were presented as to the origin of this structural fault In 1948 however Allison [ ] showed that none of these theories could be justi fied. He explained the disease as a con genital hiatus hernia, and the shortening of the oesophagus as a secondary phe nomenon. He demonstrated how the sliding hiatus compromises the function of the cardia, and how the subsequent reflux oesophagitis leads to cicatricial lesions in the oesophagus, with shrinkage shortening and fixation.

Allison s theory [2 3] was confirmed in a study by Husfeldt *et al* [9]. This in cludes a total of 24 patients with hiatus hernia and secondary shortening of the oesopaghus. Ten of these patients under went transthoracic operation, with the intention of bringing the cardia below the diaphragm All these patients were less than one year old on first admission to hospital. Surgical therapy was not con sidered indicated in 14 of them.

A follow-up of the 10 patients who were operated on showed that in eight of them where peroperatively the cardia could easily be brought down below the dia phragm, seven were thriving, being com pletely without symptoms, while one had recurrence In the two cases where the cardia could not be brought below one patient had been treated by resection with good results, while the other patient whose cardia had been loosened without being brought down into the abdomen, still suffered from regurgitation and recur rent haematemesis

Of the 14 infants who had not been operated on, one had died and two could not be traced. None of the other 11 was completely free from symptoms, only two of them had improved, while nine were either unchanged or their condition was worse than at first examination.

It was therefore concluded that the children should have been operated on at an early stage before the development of severe changes in the wall of the oeso-phagus and the perioesophageal tissue which made it difficult to fix the cardia below the diaphragm

In the present material the dominating symptom was vomiting, while in addition there was regurgitation and failure to thrive. Only 3 patients had developed complications, namely stricture in one case and severe haemorrhagic oesophagitis in two cases. The fact that 2 patients with haematemesis and melaena had to be operated on is in full agreement with previous communications, which point out that severe erosive haemorrhagic oeso-phagitis only rarely responds t medical treatment.

The present material definitely supports an ttitude of reserve t wards operative therapy of hiatus hernia in infants as 19 out of — infants became completely symp-t mfree as result of conservative treat ment Operative therapy is indicated only in the case f severe complications. The primary task must be to make the diag nosis s early as possible by means of

radiological examination, and to counteract the development of severe oesophagitis by maintaining the infant in a sitting position as soon after diagnosis as possible and as effectively as possible.


## Summary

Twenty two infants with hiatus hernia were treated conservatively by the method of Carré. Three patients with severe complicating oesophagitis did not react to treatment The other 10 patients became well, and on follow up about 3 years later showed no subjective symptoms although 8 of them still had a radiologically demonstrable hernia.

It is maintained that operative treatment can usually be avoided, if conservative treatment, i.e. keeping the infant in a sitting position can be instituted as early as possible


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# Trisomy G/Normal Mosaics in Non mongoloid Mentally Deficient Children

by H. H. VAN GELDEREN, J. L. J. GAILLARD and A. SCHABERG

In a cytogenetic study of patients from an institution for mentally deficient children a large number of chromosomal aberrations have been found among cases selected because of multiple congenital anomalies, retarded growth and without other explanation for mental deficiency [9, 10]. Among the patients with chromosomal abnormalities there were 8 cases of trisomy G mosaicism. Patients with clinically undoubted Down's syndrome have not been included.

The purpose of this paper is to describe and discuss the cytogenetical and clinical findings in these cases of trisomy G mosaicism.

## Methods

Cells of peripheral blood were cultured according to the slightly modified, short term culture technique as described by Moorhead & Nowell [23]. To spread metaphase chromosomes Rothfels & Siminovitch [27] air drying technique was used. Alkaline phosphatase activity in leucocytes has been estimated according to the histochemical method of Kaplow [16]. Lobe count was calculated by taking the mean of the number of lobes of 200 granulocytes.

## Case Material

The cases with trisomy G mosaicism have been detected during a cytogenetical survey of a selected group of children from an institution for mentally deficient children. Description of this survey is published elsewhere (van Gelderen et al. [10]). A ninth case investigated in the University Children's Department has been added. None of the children were ill at the time of investigation and only one was on drug treatment (phenothiazine). Their age ranged from 1–18 years. Groups for comparison of leucocyte alkaline phosphatase activity and lobe counts consisted of typical cases of Down's syndrome and mentally deficient children with brain damage of either perinatal or postnatal origin, all from the same institution.

## Results of Cytogenetical Investigation

The chromosomal analyses of the nine cases of trisomy G mosaicism have been listed in Table 1. Except in case 7 the cells with 45 (or less) chromosomes did not reveal a constant pattern upon analyses, in contrast with the cells with 47 chromosomes which virtually always showed an extra small chromosome (Fig. 1). In case 7 there is probably also an XO cell line which fits in with the fact that the now 17 year-old patient has primary amenorrhoea, sex-chromatin is present. In case 8 the extra chromosome is somewhat larger than those of group G and metacentric (Fig. ) We consider it as a possible G/G

TABLE 1 *Cytogenetic analysis of trisomy G mosaics.*

| Case | Cells counted | Number of cells with < 46 chromosomes | 46 chromosomes | 47 chromosomes | > 47 chromosomes | Interpretation | Cultures |
|---|---|---|---|---|---|---|---|
| 1 | 60 | 9 | 31 | 18 | 2 | Extra G chrom. in ca. 36 % | 1 bl. cult. |
| | 200 | 40 | 145 | 15 | 0 | Extra G chrom. in ca. 6½ | 3 bl. cult |
| 3 | 200 | 32 | 149 | 18 | 1 | Extra G chrom. in ca. 9 | 3 bl. cult. |
| 4 | 100 | 15 | 6 | 9 | 0 | Extra G chrom. in ca. 9 | bl. cult |
| 5 | 100 | 5 | 88 | | 0 | Extra G chrom. in ca. 7 | 1 bl. cult. |
| 6 | 150 | 5 | 1_5 | 20 | 0 | Extra G chrom. in ca. 13 | bl. cult. |
| 7 | 100 | 17 | 65 | 15 | 0 | Extra G chrom. in ca. 15 also XO/XX mosaic | 1 bl. cult. |
| 8 | 200 | 30 | 108 | 61 | 1 | Extra G chrom. in ca. 30 | 3 bl. cult. |
| 9 | 150 | 19 | 0 | 61 | 0 | Extra G chrom. in ca. 40 | bl. cult. |
| | 100 | 14 | 57 | 29 | 0 | Extra G chrom. in ca. 30 | 1 skin cult. |

Blood culture

translocation though a deleted super numerary other chromosome cannot be excluded.

Case 9 warrants further description. In this child the extra chromosome is a very small one (Fig 3) like a Philadelphia chromosome It is also present in skin cells and there are no signs of leucaemia whatsoever

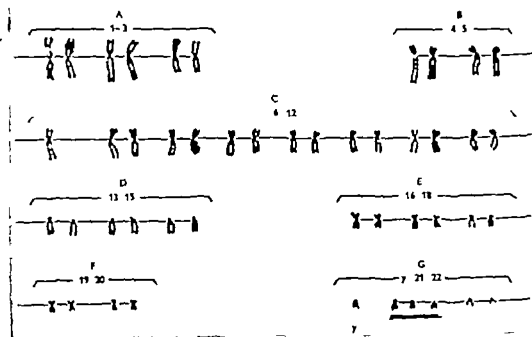



Fig. 1 Karyogram of cell with 47 chromosomes showing trisomy G (case 1).

Fig. 2. Karyogram of cell with 47 chromosomes, showing the extra, somewhat larger chromosome (case 8).

Results of Clinical Investigations

T ble 2 lists the main clinical features of the 8 patients from the institution and a ninth case from the Children s Depart ment.

The table demonstrates th variability of the features, though most of the children do show a number of the malformations usually present in Down s syndrome Dermatoglyphics are mostly not typical (Log index [7] within mongolian range in two patients only) but high axial triradius is found in 4 of 8 cases Short stature (usually with normal or only slightly retarded bone-age) is always present as is severe mental deficiency These however belong to the selection criteria

Fig 4 show the faces of seven of the patients. They do not resemble each other

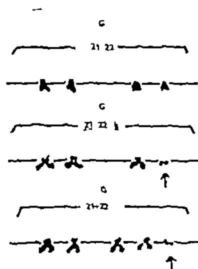


Fig 3 The G chromosomes of case 9 Upper line from normal cell, second line showing the very small hromosome from cell with 46 chromosoms and third line showing the extra small chromosome from cell with 47 chromosomes.

Finley *et al* [6] recently reviewed a total of 30 published cases. Richards *et al* [25] have collected 10 surveys of cytogenetical investigations of patients with Down s syndrome (including one performed by themselves) covering a total of 784 patients. Among these there were ?.5 % trisomy 21 mosaics.

On the other hand a few patients with trisomy G mosaicism have been reported, who did not resemble Down s syndrome and therefore were considered to be tri somy 22 mosaic Turpin & Lejeune [33] discuss these cases and conclude that this diagnosis remains speculative

Case 9 differs from the other cases because there is a very small chromosome (fragment) both in part of the trisomic and of the euploid cells. Such a small chromosome has been reported in mentally deficient children with multiple con genital anomalies, sometimes resembling Down s syndrome [4 14 10] and in a child with anal atresia and coloboma (29) Hall [1 ] has found two male infants with Down s syndrome who also showed an extra Philadelphia like chromosome An extra small autosome resembling a deleted E chromosome has been described in a severely malformed young child [8] Deletion of a G chromosome [?0 ?6] has been (somewhat fancifully) called antimongolism The features of these children suggest a rather typical phaenotype, in some ways the opposite of mongoloid features. Combination of trisomy XXY/XY mosaicism and deleted G has also been described [?4]. Our patient combines to a certain extent the cytogenetic abnormalities of patients with chromosome G deletion and those with trisomy for a very small chromosome A more detailed description, including family study to try to establish the origin of the abnormal chromosomes, is under way

We are not aware of any other study of mentally deficient non mongoloid patients which also revealed a number of trisomy G mosaics If we followed the reasoning of the authors referred to earlier we might have considered four of our cases (who do resemble Down s syndrome to a certain extent) to be trisomy ?1 mosaics. In the others the diagnosis would remain trisomy G (or 22 ?) mosaicism.

There are however a few other arguments suggesting that our cases might be examples of trisomy 21 mosaics. The maternal age at birth (Table 2) is higher than average in our country The same is true for the reported cases of trisomy ?1 mosaicism [25] notwithstanding the fact that a number of these mosaics have been detected in surveys of patients with Down s syndrome born from young mothers

The results of the estimation of leucocyte alkaline phosphatase activity in our cases suggests that the extra chromosome might well be a ?1 chromosome As Table 3 has shown, the enzyme activity is increased considerably The only other report of leucocyte alkaline phosphatase activity in trisomy G mosaics we have encountered is that of Kontras *et al* [18] who also mentioned high values in cases.

Study of patients with mosaicism for trisomy 1 may give some check upon claim about the relationship between characteristics of Down s syndrome and genes lying upon the extra chromosome Several of these claims have been made as e.g ABO genes (refuted by Kaplan *et al* [15]) alkaline phosphatase in leucocytes

TABLE 4. *Mean lobe count in patients with trisomy G mosaicism Down s syndrome and controls*

| | Number | Mean lobe count | Range | S.D |
|---|---|---|---|---|
| Trisomy G mosaics | 8 | 2.25 | 1 76–_82 | 0.38 |
| Down syndrome | 7 | 1 75 | 1.51–2.12 | 0.25 |
| Controls | 10 | 2.03 | 1.79–3.37 | 0.36 |

and galacto-1 P uridyltransferase As to the latter enzyme Brandt *et al.* [ ] suggested that the locus for the corresponding gene is located upon the 21 chromosome as the enzyme activity in whole blood in Down s syndrome had been found to be about one and a half that of normal people. Hsia *et al.* [13] showed that this was true for leucocytes only but not for erythrocytes. Mellman *et al.* [22] reported that acid phosphatase and G 6P.D activities were also increased in leucocytes of patients with Down s syndrome while the increase of activities of the three enzymes correlated mutually All this and our findings of high enzyme content in mosaics suggest that increased enzyme activity is not a gene dosage effect but somehow related to leucocyte abnormalities in Down s syndrome

Since 1947 it has been known [32] that leucocytes in Down s syndrome are different because the lobe count (Arneth count) is much lower than in normal people From Table 4 it seems clear that our cases of trisomy G mosaicism how a decreased lobe count, about intermediate between that of Down s syndrome and normal. This also suggests that the extra chromosome might be a 1 chromosome. It would even fit with the hypothesis of direct relationship between lobe count and chromosome 21 material It seems desirable to determine lobe counts in other trisomy G mosaics as only study of a larger number of cases can confirm our results and might show an inverse correlation between degrees of mosaicism and lobe counts

About the mechanism causing mosaicism not much can be said with certainty

If we for the moment, assume the extra chromosome in our patients to be a 21 chromosome, the maternal age distribution at birth suggests that mosaicism occurred in an originally trisomic zygote Combining data of our 8 patients with those of the 18 mosaics from the surveys collected by Richards *et al* [_5] a distribution of maternal age at birth is obtained as demonstrated in Table 5. This table should be evaluated with caution. The distribution of maternal ge in all births differs from one country to another and the patients come from several western countries For a rough comparison the maternal age distribution t birth in 1950 in the Netherlands is given.

It seems lear all the sam that this small sample of trisomy G mosaics shows a maternal ge dependency comparable to that of Down syndrome the more so because som bias to younger maternal ages still xists in the material. Among 110 patients with Down syndrome born from young mothers, Turpin *et al.* [33] found no ase with mosaicism at all.

TABLE 5 *Maternal age at birth of 27 trisomy G (21) mosaics and regular trisomy 21 (partly from Richards et al [25]*

| Maternal age at birth | Mosaics | Regular tris. 21 | All live births* |
|---|---|---|---|
| 30 years and younger | 11 – 41 % | 232 – 32 % | 50 % |
| 31–35 years | 4 – 15 % | 114 – 15 % | 28 % |
| Older than 35 years | 12 – 44 % | 390 – 53 % | 22 % |
| | Total 27 | 736 | *217 000 |

The Netherlands, 1950.

The age distribution of the mothers shown in Table 5 indicates that the hypothesis of loss of the extra chromosome in originally trisomic zygotes seems more likely than the assumption of non-disjunction in the early divisions of a normal zygote.

The literature about trisomy 21 mosaicism indicates a frequency of about 2 per cent of that of Down's syndrome [15]. The institutionalized population in which 8 of our cases were detected also contains 80 patients with typical Down's syndrome which suggests that trisomy G mosaicism is much more common than would be deduced from the literature.

## Summary

A cytogenetic study of mentally deficient children with multiple congenital malformations not including typical cases of Down's syndrome resulted in the finding of 8 patients with trisomy G mosaicism. They belong to an institutionalized population which also contain about 80 cases of Down's syndrome. The frequency of this type of mosaicism therefore seems to be much higher than the reported frequency of trisomy G (21) mosaics found in patients with more or less typical Down's syndrome.

The question whether the extra G chromosome is a 21 or 22 chromosome cannot be answered yet. The maternal age distribution, high leucocyte alkaline phosphatase activity and decreased lobe count however suggests that chromosome 21 is involved.

The clinical picture ranges from a resemblance to Down's syndrome up to complete different features. The literature about trisomy G (21) mosaicism is reviewed and the influence of patient selection discussed.

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REVIEW ARTICLE

# Primary Arteritis (Pulseless Disease) in Korean Children

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Since Takayasu [19] described a case of pulseless disease with retinal changes in 1908, many similar cases have been reported in literature especially from the Oriental countries. Before aortography was available much attention was paid to the absence of pulse and the presence of ophthalmological findings, as well as to the cerebral symptoms secondary to occlusive lesions in the aortic arch. However the increasing use of aortography in the diagnosis of this disease has frequently shown the presence of atypical coarctation of the abdominal or the thoracic aorta The renal arteries are also commonly involved. Cardiomegaly and hypertension are the most common findings especially in children. Pulseless disease has become a subject of great interest from the viewpoint of vascular surgery

During the past five years we have encountered ten cases of pulseless disease or primary arteritis in Korean children. Five of them (cases 1 4 5 6 and 7) have already been reported in detail [5 10]. All ten cases will now be summarized, followed by a brief review of the literature

## Review of our Ten Cases

During the period from January 1962 to December 1966 10 cases with primary arteritis or pulseless disease have been observed in the pediatric departments of the National Medical Center (7 patients) and the Seoul National University Hospital (3 patients) The diagnosis was mainly based upon the aortographic findings.

A short survey of the 10 patients is given in Table 1 All were under 1 years of age the youngest 5 years and 3 months; 8 were females.

### *Clinical manifestations*

The relevant clinical manifestations are summarized in Table 2.

The preceding symptoms were dyspnea in 8 patients, edema or puffy face in 5, palpitation headache and general weakness in 3 and intermittent claudication in one The duration of symptom before examination varied from 2 months to 3 and a half years.

The main clinical signs were hypertension and cardiomegaly in 8 and 10 patients respectively A bruit ver the abdomen was heard in 7 patient and

TABLE 1 *Summary of the ten patients*

| Cases | Sex | Age in years | Chief complaints | Duration of symptoms | Duration of follow-up | Present stat |
|---|---|---|---|---|---|---|
| 1 T K. U | F | 5[illegible] | Dyspnea, general weakness | 2 mo. | 45 d. | Expired[a] |
| 2. B. J Y | F | 6[illegible] | Dyspnea, cough, edema, oliguria | 2 mo. | 2 y | Alive |
| 3. Y A. K. | F | 9[illegible] | Dyspnea, cough, edema | 6 mo. | 1[illegible] y | Good |
| 4. K. S. L. | F | 10 | Headache puffy face | 16 mo. | 10 mo | Expired |
| 5. O J H. | F | [illegible] | Dyspnea, palpitation | 1 mo | 4 y | Excellent |
| 6. Y A. K. | F | 11 | Dyspnea, puffy face palpitation | 3½ y | 3 y 3 mo. | Worse |
| 7 K. I. K. | M | 12 | Headache hypertension | >1 y | 2 y 11 mo. | Better[a] |
| 8. Y S. C. | M | 7[illegible] | Dyspnea, edema, palpitation | 4 mo. | [illegible] mo. | Expired[a] |
| 9. B. J L. | F | 11[illegible] | Dyspnea, general weakness | [illegible] mo. | 2 mo. | Expired |
| 10. J H. K. | F | [illegible] | Dyspnea, general weakness, headache, claudication | 48 d. | 10 d. | Unknown |

Case 1 By pass graft, Case nephrectomy right; Case 8 by-pass graft.

absent or weak radial pulse was demonstrable in 6 patients. The ophthalmoscopic findings were normal in all patients except in case 7 which presented grade III hypertensive retinopathy The point of maximal cardiac impulse was broad and heaving in character and was displaced downward and laterally in all 10 cases. There were no differences between the circumferences of respectively the arms or the legs in any case

*Laboratory findings*

As shown in Tables 3 and 4, the laboratory data were generally unremarkable.

Hematologic findings such as the hemoglobin value sedimentation rate leukocyte and eosinophile counts did not show any remarkable changes.

The total serum protein and the electrophoretic fractions were also within normal limits in 9 patient examined

TABLE 2 *Clinical manifestations*

| Chief complaints | No. | Clinical signs | No. |
|---|---|---|---|
| Dyspnea | 8 | Cardiomegaly with broad and heaving PMI[a] | 10 |
| Edema or puffy face | 3 | Hypertension | 8 |
| Palpitation | 3 | Bruit over abdomen | 7 |
| Headache | 3 | Absent or weak pulse | 6 |
| General weakness | 3 | ECG LVH or LAD | 9 |
| Intermittent claudication | 1 | Positive Mantoux test | 9 |

PMI point of maximal impulse

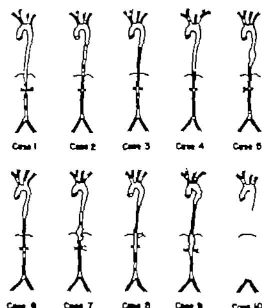



Fig 1 Schematic drawing of the aortographic findings.

Proteinuria was found in 4 patients. The concentrations of blood urea nitrogen (BUN) and creatinine were normal except in Case 8 where the creatinine value was 2.0 mg/100 ml. L. E. cells were not found in 6 cases investigated, the test was not made in the remaining 4 cases. The anti-streptolysin O titer (ASOT) was not elevated Serologic test for syphilis (VDRL) was negative in all 10 cases; the Mantoux test was positive in 9 of the 10 patients.

Chest x ray examination almost invariably disclosed cardiomegaly and pulmonary congestion. Intravenous pyelography (IVP) was not carried out in a few patients; however the renogram could be seen on the aortographic examination. The electrocardiogram (ECG) showed patterns of left ventricular hypertrophy with or without left axis deviation in 9 of the 10 cases. The blood pressure readings ECG and roentgenologic chest findings are given in Table 5



Fig. 2. Case 1 Narrowing of abdominal aorta and stenosis of both renal arteries.

TABLE 3 *Laboratory data—I*

| Case | Hgb g | SR mm/hr | Leukocyt per cmm | Eosinophils % | Total protein, g | Albumin | Alpha 1 globulin | Alpha 2 globulin | Beta globulin | Gamma globlin |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 0 | 19 | 10,400 | | 7 12 | 3 73 | 0.33 | 0.46 | 0 87 | 1 74 |
| 2 | 11 7 | 3* | 11,950 | | 5 96 | 2.90 | 0.37 | 0.70 | 0 75 | 1.31 |
| 3 | 1_1 | 3 | 8,300 | 1 2 | 7._5 | 3 9 | 0.22 | 0 73 | 0.90 | 1.39 |
| 4 | 10 9 | 5–23 | 7,000 | | 6.9 | 4 14 | 0.30 | 0.52 | 0.83 | 1 42 |
| 5 | 13 6 | 10 | 8,100 | 3 | 5.8 | 3.95 | 0 15 | 0 44 | 0 59 | 0.67 |
| 6 | 13 7 | *0 | 8,500 | 1 | 7.3 | 3 80 | 0.30 | 0.40 | 0.60 | 1 90 |
| 7 | 14 0 | 4–16 | 6 400 | 2 | 7 0 | 2 69 | 0 42 | 0.65 | 1 12 | 2.12 |
| 8 | 10.7 | 8 | 7,600 | 5 | 6 6 | 4 0 | 0_ | 0.6 | 0 8 | 1 6 |
| 9 | 1_7 | 7 | 13,200 | 1 | 6.92 | 3.06 | 0.50 | 0.85 | 0 85 | 1.56 |
| 10 | 11.8 | 18 | 9 900 | — | — | — | — | — | — | — |

TABLE 4. *Laboratory data—II*

| Case | Urine | BUN mg | Creatinine mg | LE cell | ASOT unit/ml | VDRL | Mantoux test |
|---|---|---|---|---|---|---|---|
| 1 | (−) | 9.3 | not done | not done | not done | (−) | (+) |
| | (−) | 11 | not done | not done | not done | ( ) | (+) |
| 3 | Albumin(+) RBC 15–20 | 10.6 | not done | not done | 100 | (−) | (+) |
| 4 | Albumin(+) | not done | 0.7 | (−) | 100 | (−) | (+) |
| 5 | (−) | not done | 0.7 | (−) | 100 | (−) | (+) |
| 6 | (−) | not done | 0.6 | ( ) | 100 | (−) | (+) |
| 7 | Albumin(+) | not done | 0.9 | (−) | 35 | (−) | (+) |
| 8 | (−) | not done | 1.0 | (−) | 50 | (−) | (−) |
| 9 | Albumin(±) | not done | 0.6 | (−) | 160 | (−) | (+) |
| 10 | (−) | not done | not done | not done | not done | (−) | (+) |

*Aortographic findings*

Aortography was performed in all 10 cases. A schematic drawing of the findings is shown in Fig. 1 and the site of lesion given in Table 6.

As summarized in Table 7 the abdominal aorta was most commonly involved (8 cases), next were the subclavian artery (7 cases) thoracic aorta (6 cases) and the renal artery (5 cases). Illustrative aortographic pictures are shown in Figs 2 and 3.

*Clinical course*

The present state of the patients and the duration of the follow-up are given in Table 1

Surgical procedures were applied in 3 cases. Case 1 revealed clinical signs of hypertensive encephalopathy and underwent a surgical by pass procedure but she expired 30 hours after the operation. In Case 7 right nephrectomy was done and the blood pressure decreased to 140–160/100–120 mm Hg from a level

TABLE 5 *Blood pressure, ECG and chest x ray findings*

| Case | Blood pressure mm Hg: Rt arm | Lt arm | Rt leg | Lt leg | ECG | Chest X ray findings: Cardiomegaly | Pulm. congestion |
|---|---|---|---|---|---|---|---|
| 1 | 162/90 | ( ) | 140/100 | 138/100 | LVH* | ( ) | ( +) |
| | Weak | ( ) | 100/74 | 100/78 | LAD | ( ) | (+ +) |
| 3 | 183/100 | ( ) | 130/90 | 130 80 | LVH | ( +) | ( ) |
| 4 | 190/110 | ( ) | ( ) | 190/110 | LVH | ( ) | ( ) |
| 5 | 90/78 | 118/90 | 90/85 | 85/63 | Normal | ( ) | ( ) |
| 6 | 185/90 | 120/110 | 120/100 | (−) | R & LVH | ( ) | ( +) |
| 7 | 220/160 | 220/160 | 240/135 | 240/135 | LVH | ( ) | ( ) |
| 8 | 170/140 | 170 140 | ( ) | ( ) | LVH | ( ) | ( ) |
| 9 | 155/110 | 170/110 | 150/110 | 150 110 | LVH | ( ) | (+ + ) |
| 10 | 190/110 | 190 140 | ( ) | ( ) | LVH | ( ) | ( ) |

LVH Left ventricular hypertrophy LAD: Left axis deviation
(−): Unobtainable ( ) Slight degree ( ): Moderate degree ( ): Severe degree.



Fig. 3. Case 8 N rrowing of thoracic aorta.

of 220/100 mm Hg before surgery Case 8 showed remarkable but only temporary improvement after by pass surgery the blood pressure was 170/140 mm Hg on the arms but unobtainable on the legs before operation After the surgical inter vention it was 110/90 mm Hg on the arms and 130/? mm Hg on the legs. She expired one year after the operation.

Medical treatment with digoxin, diuretics, steroids and vitamin B complexes was tried in the remaining 7 cases, but without appreciable benefit. Case was not followed up but we have been told that she is still alive without complaints. In Case 3 the pulse became palpable in the left arm after medical treatment. Case 4 expired at home after discharge Case 5 the only one without hypertension,

TABLE 6. *Site of lesions (diagnosed by aortography)*

| Case | Carotid artery | Subclavian artery | Thoracic aorta | Abdominal aorta | Renal artery | Terminal aort | Other arteries |
|---|---|---|---|---|---|---|---|
| 1 | ( ) | (−) | ( ) | Narr | narr | ( ) | Sup mesent a. occlusion |
| | l ( ) | l occl | | | l narr | | |
| 2 | ( ) | narr | ( ) | Narr | ( ) | ( ) | ( ) |
| | l ( ) | l occl. | | | l ( ) | | |
| 3 | ( ) | (−) | Irreg | Irreg | occl. | ( ) | ( ) |
| | l (−) | l occl. | | | l ( ) | | |
| 4 | ( ) | ( ) | ( ) | Narr | narr | Narr rt il. artery | ( ) |
| | l narr | l occl. | | | l narr | | |
| 5 | (−) | narr | Dilated | Narr | ( ) | ( ) | ( ) |
| | l ( ) | l ( ) | | | l ( ) | | |
| 6 | ( ) | ( ) | Narr | Narr | r ( ) | Narr lt. il rtery | ( ) |
| | l ( ) | l narr | | | l ( ) | | |
| 7 | ( ) | ( ) | ( ) | Irreg. | r occl | ( ) | ( ) |
| | l (−) | l ( ) | | | l narr | | |
| 8 | ( ) | ( ) | Narr | (−) | ( ) | ( ) | I tercostal occlusion |
| | l ( ) | l ( ) | | | l ( ) | | |
| 9 | ( ) | ( ) | Narr | Narr | ( ) | Narr l aa. | Rt vertebral and mamm. aa. change |
| | l ( ) | l narr | | | l narr | | |
| 10 | ( ) | ( ) | Narr | ( ) | ( ) | ( ) | ( ) |
| | l ( ) | l ( ) | | | l ( ) | | |
| Total | 0 | r 2 | 6 | 8 | 4 | 3 | 3 |
| | l 1 | l 6 | | | l 4 | | |

Irreg irregular Narr narrowing Occl.: occluded right l left.

TABLE 7 *Site of arterial lesions*

| Site of lesion | No. of lesions | No. of patients |
|---|---|---|
| Carotid: Right | 0 | |
| Left | 1 | 1 |
| Subclavian: Right | 2 | |
| Left | 6 | 7 |
| Thoracic aorta | 6 | 6 |
| Abdominal aorta | 8 | 8 |
| Renal: Right | 4 | |
| Left | 4 | 5 |
| Terminal aorta | 3 | 3 |
| Other arteries | 3 | 3 |

is doing well and enjoys ordinary life Case 6 is gradually getting worse and is still attending the out patient-clinic because of congestive heart failure. Case 9 developed generalized convulsions, attributed to diffuse cerebrovascular insufficiency Her symptoms became worse and she finally expired. The present state of Case 10 is unknown.

## Review of the Literature and Discussion

The first description of the *aortic arch* or *pulseless syndrome* with symptoms resulting from narrowing or occlusion of the large brachio-cephalic vessels appears to have been given by Davy in 1839 [4] In 1908 at a meeting with Japanese ophthalmologists, Takayasu [19] reported some peculiar and previously unknown changes in the eye-ground of a patient. In commenting on Takayasu's case Onishi first correlated the absence of radial pulses with the ocular manifestations of the disease [7] Since then many cases of pulseless disease have been reported from all part of the world. In 1926, the histology of an aortic arch arteritis in a young woman was described by Harbitz and Raeder Martorell & Fabre [1 ] published a paper in 1944 which is generally acknowledged as the first adequate description of the clinical picture associated with obliteration of the great trunks of the aortic arch. Ross & McKusick [16] credit Gordon Murray with carrying out the first arterial reconstruction of the aortic arch vessels in 1930 but the first published case report is that of Davis *et al.* in 1956 [3].

Many different terms have been given to this condition. pulseless disease pulseless syndrome Takayasu's disease Takayasu's syndrome Takayasu-Onishi's disease Martorell's disease aortic arch syndrome aortic syndrome "middle aortic" syndrome aortic bifurcation syndrome; young female arteritis of the aortic arch, brachiocephalic arteritis, aortic arch arteritis; primary arteritis, idiopathic arteritis; aortitis syndrome panaortitis syndrome primary aortitis, typical coarctation of aorta, reversed coarctation, reversed coarctation syndrome. Among these the authors prefer the term primary arteritis [5 10].

The disease occurs at any age but very rarely in childhood. According to Ueda *et al* [20] who reviewed 321 cases (Table 8) the peak incidence was noted at the age between 20 and 29 years, with female predominance (8 1) Among these 321 cases, only 5 (4 females and 1 male) were below the age of 10 years. Geographically the disease predominates in the Orient Up to 1957 90 cases were reported [8] and 53 of these were from Japan (Table 9) Up to the end of 1960 approximately 30 cases had been reported in the Korean literature including the present 10 cases.

The etiology of primary arteritis still remains unknown. A positive tuberculin

TABLE 8 *Age and sex distribution* [20].

| Age (years) | Males | Females | Total |
|---|---|---|---|
| 0–9 | 1 | 4 | 5 |
| 10–14 | | 11 | 13 |
| 15–19 | — | 46 | 46 |
| 20–24 | 1 | 73 | 85 |
| 25–29 | 6 | 55 | 61 |
| 30–34 | 3 | 54 | 57 |
| 35–39 | 4 | 21 | 25 |
| 40–44 | 2 | 9 | 11 |
| 45–49 | 3 | 7 | 10 |
| 50–54 | | 2 | 4 |
| 55–59 | 1 | 1 | 2 |
| > 60 | 1 | 1 | 2 |
| Total | 27 (11.5%) | 204 (88.5%) | 231 (100.0%) |

Average age: males 31.0, females 20.3 years.

reaction has been noted by several authors. In our 10 cases, 9 were positive tuberculin reactors. However since not all patients showed a positive reaction, this finding may be related to the high frequency of tuberculosis in those communities where primary arteritis has been reported most often. Though a positive serologic test for syphilis has been present in a number of reported cases (though in none of ours) syphilis can hardly be considered as an etiologic factor The antistreptolysin O titers were within normal limits in most cases. Though a thrombotic diathesis has been considered, this phenomenon could not be demonstrated *in vitro* Environmental factors such as diet

TABLE 9 *90 cases from the literature* [8]

| Country | Number |
|---|---|
| Japan | 58 |
| Sweden | 10 |
| U.S.A. | 8 |
| Great Britain | 5 |
| Norway | 3 |
| Hungary | 2 |
| Other countries | 6 |

vitamins, general nutrition etc., were previously suspected as underlying causes [14] but the patients give no history of a common environmental condition. There has been no case of congenital malformations resulting in absence of pulses in both upper extremities Currently many attribute this disease to an autoimmune process because the general systemic manifestations resemble those seen in lupus erythematosus or rheumatic disease. However similar pathological findings were never demonstrated in primary arteritis.

*Pathology*

The pathological findings in this disease are those of a chronic arteritis involving the aorta and the proximal segments of its large branches, giving rise to segmental stenosis or occlusion. The inflammatory process affects all layers of the artery ("panarteritis") and is characterized by a marked thickening of the intima. The latter shows a pearly gray white infiltrate and is longitudinally corrugated on its inner surface. Histochemically the intimal lesions consist of mucopolysaccharide and do not have the characteristics of atheroma. The media may show areas of coagulation necrosis granuloma formation and elastic tissue degeneration. The adventitia is also thickened and may exhibit neovascularization and lymphocytic infiltration The vasa vasorum may be involved in the disease process Occasionally giant cells may be present to the extent that the process may resemble giant cell arteritis. The most common lesions are obliterative in nature but aneurysmal and saccular dilatation of the aorta have been described [11]

*Cl nical manifestations*

The clinical features vary considerably depending upon the site of the lesions [13, *1 22].

A. In involvement of the *carotid* branch, the carotid pulse may be either diminished or absent. Cerebral symptoms such as dizziness, syncope, headache aphasia seizures, transitory hemiparesis or hemiplegia, hair loss and abnormalities in the electroencephalogram may occur Ocular symptoms may develop, and these include impaired vision, transitory visual loss, cataracts, and photophobia. Venous dila tation, wreath like anastomosis, microaneurysmal formation, iris atrophy iris and conjunctival neovascularization are found on clinical examination of the eye

B In involvement of the *subclavian* branch there may simply be asymmetry of the pulse early in the course, but there is eventual progression towards a complete obliteration. Symptoms include rapid exhaustion, numbness, claudication or coldness, weak or absent pulse and un equal blood pressure in the upper extremity Sometimes, ischemic color changes may be seen.

C. Involvement of the *thoracic* aorta ("middle aortic" syndrome, supradiaphragmatic) [17] gives rise to hypertension in the upper extremities and dimin ished or absent femoral pulse Sometimes a systolic murmur is audible over the lower thoracic region

D In involvement of the *abdominal* aorta ("middle aortic" syndrome infradiaphragmatic), there is hypertension in the arms, and absent or diminished fem oral pulses, and a systolic murmur is audible over the abdomen. If a renal ar tery is affected, severe hypertension develops due to a Goldblatt mechanism If occlusion of the mesenteric artery occurs abdominal symptoms develop. Easy fatig ability coldness and claudication in the lower extremities may also occur

E. Involvement of the *aortic bifurcation* (Lerich s syndrome) causes weakness, claudication and a weak or absent pulse in the lower extremities.

F The *pulmonary* artery may be in volved [13 20].

G Various combinations of the above mentioned lesions may develop with multiple lesions.

In our 10 cases, the abdominal aorta and left subclavian artery were most fre quently involved. The next most frequent sites were the thoracic aorta and the renal arteries. This is in good accordance with the data of Sen *et al.* [17] and Paton *et al.* [15] According to Ueda *et al* [*0] who reviewed 221 cases in Japan, the order of frequency was the left subclavian and carotid artery the right subclavian and carotid artery the abdominal and the thoracic aorta. If aortography were per formed more frequently more cases with demonstrable changes in the abdominal and thoracic aorta and its branches will presumably be found.

Our 10 cases presented cardiomegaly with broad and heaving point of maximal impulse hypertension, and absent or weak pulses The main symptom of hypertension in children may be dyspnea only and dyspnea wa the chief complaint in 8 of the present 10 cases. It appears that hypertension and cardiac failure with cardiomegaly are the most common findings, and the latter has been the main cause of death in these patients. Pulse lessness is a symptom which can be easily

detected but is least important so far as the prognosis is concerned.

In the Orient, "atypical" coarctation of aorta is considered to be more frequent than the typical, congenital coarctation of the aortic isthmus. Kimoto *et al* [9] reviewed 30 cases of coarctation of the aorta in the Japanese literature up to 1960 In 18 of these cases the site of the coarctation was the abdominal aorta. Inada *et al* [6] has during 5 years, experienced 10 cases of atypical aortic coarctation but none of the typical isthmic type He concluded that these atypical coarctations are of acquired origin, due to aortitis, and that they belong to the same entity as primary arteritis. Sen *et al* [17] discovered 16 cases of this type of coarctation, while only 4 examples of congenital coarctation were observed in India Primary arteritis should be suspected in all cases of abnormal coarctation, particularly when the stenosis is in the lower thoracic or abdominal aorta [15]. Congenital aortic coarctation is more prevalent in males (2. 1) while primary arteritis is more common in females (8 1)

Ask Upmark [1] reviewed the occurrence of hypertension in Takayasu s syndrome and discussed the cause of hypertension in this condition. Hypertension was found in about half of the cases. He considered several causative factors, and emphasized the importance of the renal factor to hypertension.

Svartz Malmberg [18] reported a case of unilateral clubbing of the fingers in a 4-year-old girl, associated with obstruction of the homolateral subclavian artery of unknown etiology and stated that Takayasu s disease may be responsible for such vascular obstruction. None of our cases however showed clubbing of the fingers.

*Diagnosis*

Aortography is the most reliable diagnostic procedure It reveals the location as well as the degree of involvement Laboratory examinations are usually of little help but in some patients the sedimentation rate is elevated and changes in the electrophoretic pattern have been observed (increase of gamma globulin and alpha *-globulin)

*Prognosis*

The natural history of this disease is quite variable The prognosis depends on the degree and extent of the occlusive lesions, and also on the importance of the arteries affected. The duration from estimated onset to death varies from one and a half to twenty years [*0] Death may result from acute cerebrovascular insufficiency cerebrovascular accident cardiac failure or pulmonary edema.

Symptoms may remain unchanged for years in some cases, whereas others may progress slowly or rapidly with remissions and exacerbations

*Treatment*

No medical therapy has been able to alter conclusively the course of the disease In most cases, an extensive obstructive lesion is already present at the time the patient comes to medical attention and medical treatment is followed by little or no changes. Treatment with steroids and anticoagulants has been recommended by some authors. However steroid have to be discontinued in some cases because of progressive hypertension Chloro-

quine has also been tried, and digitaliza tion and diuretics may be necessary for control of congestive heart failure Specific anti-tuberculous treatment with supportive measures should be employed in suspected cases

Various surgical procedures have been applied to relieve the obstruction. By pass grafts or thromboendarterectomy have been tried, depending on the lesion involved. The intimal plaques are easily stripped off by endarterectomy and this procedure gives good short-term results. However if recurrent thrombosis proves serious problem, by pass grafting or resection with graft replacement may be better operations. Nephrectomy may have to be performed to lower the blood pressure in certain cases. Surgical procedures if employed correctly are ffective in re lieving the symptoms in cases with isolated lesions. However in the systemic type of this disease, surgical interventions of any kind may be of limited value The probability of recurrent occlusions after surgery may become greater in patients suffering from arteritis hence long term follow-up studies are necessary to deter mine their efficiency

## Summary

Ten cases of primary arteritis or pulseless disease in Korean children are repor ted. Aortography showed the abdominal aorta to be most commonly involved, and the subclavian artery was next in fre quency Dyspnea hypertension, cardiomegaly and cardiac failure were the most common symptoms and signs in these children. The ultimate prognosis ppeared to depend upon the degree and extent of the occlusive lesions, and also on the importance of the arteries affected. The condition should always be considered in cases of severe hypertension in children, especially in the Orient The literature is briefly reviewed

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CASE REPORT

# Arteriopathia Calcificans Infantum

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Arteriosclerotic diseases in children are rare conditions but are known in connection with idiopathic hypercalcaemia D-vitamin poisoning and in some cases of advanced kidney disorders. Furthermore universal arteriosclerotic changes appear with the so-called Arteriopathia calcificans infantum, the etiology of which is so far unknown. The disease is thought to be quite rare and only about 60 cases have been described.

In 1955 Zinchka [11] gave a detailed description of the histological changes of the disease and suggested the here used name Arteriopathia calcificans infantum (A.c.i.)

In 1946 Stryker [10] reviewing the literature found 15 cases and added 5 of his own. 1959 Moran & Becker [7] made a survey of the then known 4_ cases plus a further two from their personal experience The average ge at death of these 44 cases was 3.8 months ranging from one day to 18 months. In other published cases [1 4, 6, 9 11], all the patients have been under 1 year of age. Ivemark *et al* [5] described two cases amongst stillborn.

The clinical course has in ll cases been characterized by a rapid and fatal cardiac failure. The duration of the symptoms has varied from sudden death to some months illness and in most cases the illness lasted only a few days. Diagnosis in most cases was first made at autopsy though Nielsen [8] describes a patient a boy aged 5 months where the illness commenced with ileus, and the diagnosis was made by microscopy of the arteries to a resected part of the ileum.

That the disease may be found in somewhat elder children, and may have a more protracted course is illustrated by the following case.

## Case Report

Boy H h. F P born 14 7 1962 (Jour 137/64-65)

The family including a brother 2 years older are healthy The pregnancy birth and neo-natal period were uncomplicated, birth weight 3930 g His development was normal until he was two months old. H was then admitted to hospital with febril illness of some day duration. Temperature on admission was 39 8 C and fell in few day t about 38 C where t remained for the following three weeks From then it was normal until discharge X-ray of the heart and lungs on admission was normal, but three weeks later new X-ray showed great enlargement of the heart measuring 8.5 cm against an internal diameter of the thorax of 12.3 cm ECG showed normal conditions. H as tired on admission, pale and a little dyspnoeic w th poor ppetite but he improved

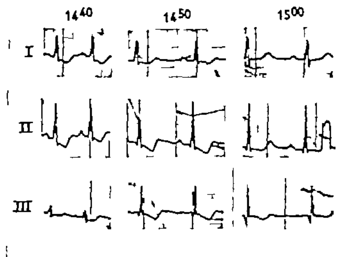



Fig 1 ECG during an attack.

slowly and after two months, as he showed no further clinical symptoms he was discharged. The heart showed a slight but not complet return t normal size

He showed no further symptoms for the next 18 months, when he about 2 years old, was admitted to the children s department For a few weeks he had had several attacks where he suddenly became extremely dyspnoeic, pale somewhat cyanotic and had to lie down. After a few minutes the symptoms disappeared and he returned to normal The attacks reappeared up to ten times a day but were only of a few minutes duration.

On admission he was thin, pale a little dyspnoeic but not cyanotic Stethoscopy showed gallop-rhythm, but no murmurs and the lungs were normal. The liver reached a *little under the umbilical line and the spleen* 3–4 cm under the left curvature. There was no oedema and no ascites. The blood pressure on admission was 125/100, and the next day normal. H was afebrile there was a slight leucocytosis, but the micro-ESR was normal. The urine was normal and contained no protein. Serum Na serum-K serum Ca, serum-creatinine and protein bound iodine were normal, but the serum glut mic oxaloacetic transaminase a little raised, 1.8 units (normal < 1 7 unit ). ECG was normal but the X ray of the heart showed a *great enlargement, measuring 10 7 cm* against thorax 17 5 cm. H was digitalized, and his condition improved after some days.

In the following period his condition was dominated by numerous attacks where he suddenly became desperately ill. The attacks often started in connect on with exertion, occasionally spontaneously and a few times when asleep. H became suddenly very rest less with screaming, but later on quiet and atonic, at first he was flushed, then ex tremely pale and slightly cyanotic with pronounced dyspnoea and laboured respiration. H was fully conscious during the attacks, which began to wear off after 5–10 minutes, nd he recovered completely in about 20–30 minutes. During the attacks there was *marked tachycardia with a pulse-rat of bout* 180/min. The attacks were always accompanied by marked hypertension with systolic blood pressure bout 170–190 mm Hg and diastolic blood pressure about 130–150. At the same time as the attack was disappear ing the blood pressure f ll and t the end of the ttack it was normal about 110/80. Between the ttacks his blood pressure was alw s found normal. The attacks ere accompanied by heavy ECG-changes with depression of the ST-segments and in ersion

of the T-waves. The changes disappeared at the end of the attack when the blood pressure was becoming normal, and a normal ECG resumed (Fig. 1).

By and by the attacks were less typical and violent, but his general condition deteriorated and he became more tired and constantly dyspnoeic.

Catheterization of the right side of the heart gave no further informations. Catheterization of the left side and angiocardiography was planned, but at the beginning of the anaesthesia the heart stopped, and the action could not be restored. His age at death was 2 years and 4 months, and he had been ill for about 5 months.

On autopsy there were apart from a large and oedematous liver and spleen only alterations in the heart. It was greatly enlarged, weighing 140 g (normal 60–70 g) and with a wall-thickness amounting to the same as in an adult in both sides. The valves and ostiums were normal, and there were no septal defects. Nor was there any supra-valvular stenosis of the aorta or stenosis of the pulmonary artery as may be seen in idiopathic hypercalcaemia. The coronary arteries were normally placed, but greatly changed, thickened and twisted and very stiff and hard. The first centimeters were about 5 mm in diameter and the lumen here about 1 mm. There were no [illegible] In the myocardium there was disseminated fibrosis, and in the septum a 2 × 2 cm large whit[e] area resembling an old infarct.

By microscopy there was found a mixture of normal and fibrous muscle structure but no fresh infarcts. The coronary arteries were greatly thickened, with marked proliferation of the intima. The media was also greatly thickened and marked calcifications were found here mostly towards the lumen, but there were no atheromatic areas. There were no changes in the main arteries or in the kidneys. The other organs and the arteries of the extremities were unfortunately not investigated.

The serum-cholesterol in blood taken from the patient after death was normal, and also in the blood from his father, mother and the older brother the serum-cholesterol was normal.

## Discussion

The diagnosis was in our case as in other published cases, first made after death. Because of the paroxysmal hypertension a phaeochromocytoma was suspected, but repeated tests of the urine for catecholamines, both in connection with the attacks and after provocation with histamine (which did not provoke an attack) showed normal values. Neither was any phaeochromocytoma found at the autopsy.

The histological changes in the coronary arteries correspond to the description of the artery changes in a. c. i. given by Zischka [11] and Reimers [9] and they were not resembling the changes found in occlusive fibroelastosis of coronary arteries in the newborn, described 1967 by Dickinson & MacMahon [3]. Other diseases such as hypercalcaemia and nephropathia were not found, and the disease must be regarded as a case of a. c. i.

The case differs in some ways from earlier cases. Firstly the patient was considerably older than the other patients described, whose age at death was on an average 4 months, and secondly the course of the disease was considerably longer than usual. Only in a few cases information of the blood pressure is given, and then it has been normal or only slightly elevated. However Gelderen et al. [4] describe a case similar to the one mentioned above. It concerns a girl aged 10 months who died after 3 months of illness with cardiac failure. She developed permanent hypertension with paroxystic elevations and ECG changes but they were not reversible as in our patient.

Nothing is known about the etiology of the disease but there is probably no connection with the arteriosclerosis of the adult thus in a.c.i atheromatous changes are never found. The ages of the patients suggest that the disease probably can start before birth, and furthermore it has been observed in two stillbirths [5]

The diagnosis is difficult to confirm during life as most cases die so quickly that there is no time for thorough investigations but in a protracted case as described here and with heart attacks quite resembling attacks of angina pectoris in adults the diagnosis should be considered and a coronary angiography might confirm the diagnosis. In the cases investigated more thoroughly at post mortem the arterial changes have appeared to be universal so that artery biopsy possibly can be of some value.

Therapeutically there is only possibility for symptomatic treatment of the heart failure with digitalis and diuretics Bickel & Janssen [1] have tried steroid treatment over a period in a patient whose illness began when he was 9 months old, and who died 5 months later There was no notable improvement in his clinical condition, and it is uncertain as to whether this prolonged duration of the disease can be ascribed to this treatment.


## Summary

A case is described in a boy who died of coronary insufficiency * years and 4 months old after 5 months of illness. The course of the disease was characterized by numerous heavy attacks of hypertension with reversible ECG changes and slowly advancing cardiac failure. Autopsy showed calcification of the coronary arteries.

At the age of two months he was ill and had over a period of two months a slight cardiac decompensation with great heart enlargement on X ray He recovered clinically and showed no symptoms for the following 18 months, but if this was the first manifestation of the arteriopathia calcificans infantum is uncertain.


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CASE REPORT

# Ruptured Aneurysm of the Aorta in a 12 Year Old Girl

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Aortic aneurysms are seldom seen in children. Approximatively 130 cases of aneurysms of the thoracic aorta in patients under 18 years of age have been reported. In children, the possible aetiologies are:

(1) cystic medial necrosis, (2) non specific inflammation of the arterial wall, resulting in a so-called mycotic aneurysm, (3) syphilis, (4) giant cell aortitis, (5) rheumatic fever (6) trauma.

A satisfactory classification of these cases cannot be achieved, partly because of insufficient data in the literature. The first two groups are the largest [2, 4 5, 6, 7 8 10 1., 13 14, 17 1 23 .4 26]. A few cases of congenital syphilitic aneurysms have been reported, none of which, however belongs to the last decades [1 6]. Three aortic aneurysms caused by giant cell aortitis have been described [9 .5]. The aetiological importance of rheumatic fever has been a subject of discussion [14]. Calvin et al. [6] reported 11 cases in patients under 18 years of age. Only a few traumatic aneurysms of the aorta have been described in children [6, 15], but the number may grow with the increase in traffic accidents.

## Case History

The patient was a 12 year-old girl from a healthy family. She had previously had chickenpox and measles and, in connection with attacks of common cold, she had sometimes had a persistent distressing cough but had otherwise been well. No hyperflexibility of the joints or impairment of sight had been noticed. In February 1965 she contracted influenza with a persistent fever and cough, and was subsequently very tired. A bacterial culture from the throat was negative. There were no joint symptoms. She had sometimes had precordial pain on exertion and also post prandially. Examination of the heart on June 21, 1965, revealed pathological changes with a continuous apical murmur. The electrocardiogram was normal except for notched P waves in leads II and III. An X-ray of the chest showed a large bulge at the site of the ascending aorta. An aneurysm of the sinuses of Valsalva or some kind of arteriovenous aneurysm was suspected.

On July 5, 1965 the patient was admitted to the Department of Pediatrics in Lund for a more thorough examination. The month before admission she had felt well, and had had normal physical activity (been swimming, etc.) During a routine test in which the girl performed work at the lowest intensity—pulse rat 112/minut and ECG unchanged—she suddenly fell off the bicycle and was deeply unconscious. No respiratory movements and no peripheral blood pres-



Fig. 1. X-ray in anterio-posterior ( ) and lateral (b) view taken few day before death, showing marked broadening of the middle part of mediastinum due to dilat tion of the ascending aorta.

sure could be registered but the heart was still beating. In spite of adequat therapeutic measures, electrocardiographic acti ity gradually impaired. A thoracotomy was performed, showing haemopericardium and cardiac arrest. On opening the peri cardial sac, the wall of the aorta was found to be totally ruptured. It was sutured pro-isionally but cardiac activity did not return

## Autopsy

The girl was well developed for her age tall and slender with normal muscular development. There were no skeletal deform ities and no arachnodactyly. The peri cardial sac was opened and the common pleuro-pericardial cavity was seen to be filled with fluid and clotted blood. The heart, which weighed 250 g, showed no hypertrophy of the left ventricle. The valves were all normal in circumference. The valve cusps were normal in appearance and mobile. No structural changes were seen in the heart.

The ascending aorta howed a fusiform dilatation, starting a few mm above the coronary ostia and ending t the origin f the innominate artery. The aneurysmal was about the size of an orange and on its posterior wall t showed a 3-4 cm long, blique, sutured tear. The wall of the aneurysm was bout twice as thick as and less elastic than the aortic wall distal to the innominat artery. The intima had a slightly roughened and granulated surface with a yellowish brown discoloration. There was



Fig. 2. C ...wing



Fig. 3. The ascending aorta shows extensive destruction of the media and slight adventitial fibrosis. Elastic stain ×7

sharp limit between the aneurysmal sac and the normal part of the aorta. Intimal changes were also seen around the right coronary ostium which was normal in width; the other coronary arteries were unremarkable. The adventitia of the aneurysm appeared fibrous with brownish discolouration. The thoracic aorta distal to the arch, as well as the abdominal aorta and other vessels were completely normal.

In addition the autopsy revealed pulmonary oedema, general congestion, and Meckel' di erticulum.

## Microscopic Examination

Several sections of the aortic wall were examined after staining with hematoxylin-eosin, elastin, van Gieson, MacManus, mucicarmine colloidal iron, and Alcian blue. Histologicall the thickening of the aneurysmal wall, which was sharpl demarcated from the normal part of the aorta, was seen to be due mainly to disintegration of the media where both elastic and muscular fibres howed extensive degeneration or were irregularly displaced. There were large areas of mucoid substance which did not stain with the MacManus and mucicarmine techniques but stained blue with colloidal iron and Alcian blue. In sections treated with testicular hyaluronidase for 30 minutes t 37°C only a faint blue colour



Fig. 4. Pronounced cystic medial necrosis in aneurysmal wall. In many areas hardly an elastic membranes remain. Elastic stain ×6

ppeared after staining with colloidal iron. In the mucoid substance there were sparse stellat cells and a few lymphocytes. In some places h aline connective tissue was seen. In association with these changes there as an increase in the number of vasa vasorum, the latter being thin walled, widel patent and ruptured in several areas. The ad ent tia howed a moderat fibrous thickening with sparse infiltration of both neutrophils and lymphocytes, but no giant cells or granulomas. The wall of the proximal part of the pulmonary artery showed similar but less prominent changes.

Sect ons f he heart lungs l er spleen and kidneys showed no pathological changes apart from so lern congestion.

## Discussion

The patient was 1 -year-old girl, who on light physical exertion had a rupture of an aneurysm of the ascending aorta

which had been diagnosed a few weeks previously The microscopic examination showed the picture of cystic medial necrosis. The mucoid substance in the media is due to an accumulation of acid mucopolysaccharides, probably chondroitin sulfate [5 10] The degeneration of the connective tissue especially the elastic fibres, appears to be a secondary feature causing dilatation of the aorta and a dissecting aneurysm The increase in number of vasa vasorum in the destroyed media is in agreement with the findings of Gore [11]

According to the literature, cystic medial necrosis in children is often to be seen in Marfan s syndrome The cardiovascular manifestations of this hereditary disorder consists mainly of dilatation of the ascending aorta, the aortic ring and the sinuses of Valsalva, leading to aortic insufficiency sometimes dissecting aneuryam and aortic rupture Similar degenerative changes have been described in the pulmonary artery and its branches [18]. Baer *et al* [ ] were the first to associate Marfan s syndrome with aortic aneurysm. Since 1943 13 pediatric cases of aneurysm of the ascending aorta have been reported [5 6 21 ‿4 26].

McKusick [18] claims that Marfan s syndrome is inherited as a single autosomal dominant with 15% *de novo* mutations. Cardiovascular malformations are seen in 30–60% of the cases. A diagnosis of the Marfan s syndrome should not be made in the absence of ectopia lentis or alternatively a positive family history (according to McKusick).

In the case here reported there were no known hereditary factors. The girl s younger sister and brother were examined at the Pediatric Clinic, Lund, in November 1966 and found to be completely healthy Furthermore the girl had none of the ocular symptoms or skeletal deformities that are generally observed in Marfan s syndrome

Attention has also been drawn to the high frequency of cardiovascular defects (without relation to Marfan s syndrome) in young individuals with cystic medial necrosis. Skandalakis *et al* [23] found in the literature of 1928–1958 100 cases of coarctation of the aorta associated with aneurysms, 32 of which were under 20 years of age. Less than 30% of the aneurysms were mycotic and none were syphilitic. The aneurysm was usually located distal to the stenosis which seems to rule out hypertension as an aetiological factor An aortic aneurysm seldom develops before adolescence even though a coarctation has been present since birth [11] Skandalakis *et al* [23] claim that most aneurysm must be due to a congenital weakness of the vascular wall. Aortic aneurysms without coarctation but associated with multiple non-cardiovascular abnormalities have been described in infants [13 17] Medionecrosis has also been reported in an infant in combination with thrombosis of the abdominal aorta [10]

Cystic media necrosis has furthermore been seen in some other conditions e.g certain endocrine disorders such a myxoedema. Some workers have found a connection between cystic medial necrosis and pregnancy [1 20] Experimentally produced hypoxia of the aorta has caused mucocystic medial degeneration and dissecting aneurysms in animals. Lathyrism, a condition induced by feeding young rats a diet rich in seeds from the flowering

sweet pea (Lathyrus odoratus) [3], shows some features similar to the Marfan's syndrome including cystic medial necrosis.

In children two cases of so-called idiopathic aortic rupture and dissecting aneurysm have been reported since 1943. Griffith et al. [12] related a case of dissecting aneurysm of the aorta in a 14 year old boy whose mother had died from the same disease at childbirth. Coleman [7] described dissecting aneurysm, persistent foramen ovale and hypoplasia of a kidney in a 10-year-old girl. These two patients had previously been healthy and showed no other defects; nor was there any known family history. Microscopic examination of the aorta showed cystic medial degeneration in both cases. The authors discuss the possibility whether these cases might be incomplete forms of the Marfan's syndrome, the so-called forme fruste with only cardiovascular manifestations.

In the case here reported there were no cardiovascular malformations, nor were there any signs of endocrine dysfunction. The case appears to belong to the group of so-called idiopathic rupture of the aorta based on a medial necrosis of the aorta. It remains possible however that, in spite of the absence of other signs of Marfan's syndrome, it represents a forme fruste of this disease. Wagenvoort [20] claims that the aetiology of idiopathic dilatation of the aorta may vary and may include an incomplete form of Marfan's syndrome.

The symptoms that brought this patient to the doctor were not very impressive. A remarkable circumstance is the rapid course from the appearance of the primary symptom—the feeling of precordial oppression to the fatal outcome. It is conceivable that the aneurysm may have developed within this short period of time. The degree of physical effort that obviously caused the rupture itself was fairly small, and in her normal life the girl must often have exerted herself more than this.

The vascular changes in medial necrosis of the aorta usually involve the ascending aorta. Such an aneurysm should thus be accessible to surgical treatment [6]. Since the downhill course may obviously be very rapid it is important that any suspicion of an aneurysm of the aorta should call for immediate investigation.

## Summary

A case of ruptured aneurysm due to cystic medionecrosis of the ascending aorta is reported including pathologic anatomic findings. A short review of similar cases in the literature is given. The relation of cystic medionecrosis in children to the Marfan's syndrome, cardiovascular defects and idiopathic aneurysm of the aorta is discussed.

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CASE REPORT

# Acute Cerebellar Ataxia Associated with Echo Type 6 Infection in Two Children

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It is known that ataxic symptoms may occur in the course of drug intoxications [12] and of some infectious diseases such as malaria [9], whooping cough, scarlet fever poliomyelitis chicken pox rubella, mumps influenza and infectious mononucleosis [1 2, 7 8, 11 15 20 ]] Therefore ataxia must sometimes be considered only as a cerebellar symptom of an underlying disease However there are some cases presenting a rather well defined clinical picture in which ataxia is not accompanied by other apparent pathological changes This has been termed Acute Cerebellar Ataxia (A.C.A.)

According to Cornen *et al* [6] as well as Berg *et al* [ ] the clinical picture consists of sudden onset of symmetrical cerebellar signs in the absence of prodromal symptoms fever nuchal rigidity abnormalities of the cerebral spinal fluid and signs of drug intoxications. The syndrome has been generally observed in children under five years of age who frequently also present alterations in muscular tone and tendon reflexes, lethargy tremors nystagmus and transient ocular and facial paralysis. Recovery usually occurs within days or weeks after the onset of symptoms.

The viral etiology of A.C.A. has been established only in a few cases and has been attributed to infections with polioviruses [1 3 6 16], Coxsackie viruses group A [17] and B [*] and Echovirus type 9 [13]

To our knowledge cases of A.C.A. during Echo 6 infections have not been reported

## Case Reports

*Case 1*

D L. Giovanna, 3-year-old girl, was admitted on the 31st of August 1963 Her chief complaints were pain in the lower limbs drowsiness and an unsteady gait of several hours duration For two days prior to admission her parent had noticed that the child tended to fall frequently Body temperature remained normal. On admission she appeared slightly lethargic and hypertonic with brisk patellar and Achilles reflexes and normal plantar reflexes. Nystagmus and meningeal signs were absent. The standing position was possible only with wide stance Assumption of the Rom-

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berg position resulted in her falling either backwards or forwards. She was able to walk with support but her gait was unsteady wide-based and ataxic. Tremors of both hands were sometimes present. Otoscopy and funduscopy were negative. A mild pharyngitis was also noted. No other signs of infection were detectable. X rays of the skull, chest and hips were normal. A lumbar puncture revealed a normal spinal fluid. An EEG tracing showed only a slight dysrhythmia.

Subsequently the ataxic signs began to disappear gradually. Body temperature remained normal for the entire course of her illness. On the 11th of September the 10th day after the admission, the patient suddenly developed generalized weakness with vomiting, headache, and mild nuchal rigidity but all these symptoms disappeared within a few hours without therapy. During the following days the child was able to stand and walk normally; she was discharged on the 20th of September in apparent good health. EEG controls 1 month, and 3 years after the onset of the illness revealed only mild signs of dysrhythmia.

*Case 2*

D. L. Mauro, a 3½-year-old boy was admitted on the 13th of March 1964. His history revealed that six days after the administration of Sabin I oral vaccine he awoke with severe pain in the legs and refused to stand and walk. When supported, he cried, exhibited jerky movements and tended to fall. Since the 11th of March his mother had noted red spots on the child's skin. On admission the boy was in good general condition, conscious and aware of his surroundings. Standing and walking were possible only by widening his stance and his gait was clearly ataxic with cross trembling of the trunk and a definite tendency to fall. A positive Romberg was present. Muscular tone and tendon reflexes were normal.

Nystagmus and meningeal signs were absent. An erythematous exanthem was present on the face trunk and limbs. Otoscopy and funduscopy were normal. A mild pharyngitis was present. No other signs of infection were noted.

The following day the pain disappeared and the boy was able to walk alone but his gait was still rather unsteady. Fever was never observed. Two days after admission, the exanthem had completely faded and the child appeared neurologically normal. Two years later clinical examination revealed that physical and mental development were within normal limits.

## Virological Examination

Throat and rectal swabs, collected shortly after admission in both cases, were kept frozen at −20°C until inoculation was performed. Spinal fluid from case 1 was inoculated immediately following spinal puncture. All these specimens were inoculated in primary M. Rhesus or human embryonic kidney tissue cultures. The treatment of the material for virus isolation and the preparation of the cultures are described elsewhere [18].

Tubes inoculated with rectal swabs from both cases showed cytopathic effects typical of enteroviruses on the 7th to the 10th day after inoculation. Following several passages on the same cell systems the cytopathic agents had a titer of $10^{7.4}$ (case 1) and 10 (case 2) $TCD_{50}$/ml. Tissue cultures inoculated with throat swabs and spinal fluid and observed for more than 10 days did not demonstrate any degeneration.

For the serological identification of these agents, 100 $TCD_{50}$ of virus were typed in the neutralization test with 20 neutralizing doses of rabbit immune sera against poliovirus type 1–3, Coxsackie virus A type 9 and B type 1–6 and Echovirus type 1–3, 5–9, 11, 27. No neutralization with sera against the three types of poliovirus was observed. However both the isolated were completely neutralized by the mixtures containing Echo 6 antiserum and by the Echo 6 antiserum alone.

With acute and convalescent serum of both patients, neutralization tests were

TABLE 1 *Titers of neutralizing antibodies against the polioviruses some strains of Coxsackie viruses and Echo 6 virus in paired sera of patients*

| | | Strains of viruses tested | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Polio | | | Coxsackie | | | | | | | Echo | |
| Patient serum | Date | 1 | 2 | 3 | A 9 | B 1 | B 2 | B 3 | B 4 | B 5 | B 6 | E 6 prot. | E 6 isol. |
| D. L. Giovanna | 11.9.63 | 8 | < 8 | 8 | 8 | < 8 | 8 | < 8 | < 8 | 8 | < 8 | < 4 | < 4 |
| | 26.9.63 | 8 | < 8 | 8 | 8 | < 8 | < 8 | < 8 | < 8 | < 8 | < 8 | 16 | 64 |
| D. L. Mauro | 13.1.64 | 32 | 8 | 128 | 32 | 8 | 8 | < 8 | 8 | < 8 | 8 | 16 | 8 |
| | 13.4.64 | 32 | 8 | 128 | 32 | 8 | 8 | < 8 | 8 | 8 | < 8 | 256 | 128 |

performed employing scalar four-fold dilutions of the sera and 100 $TCD_{50}$ of the prototype strains of poliovirus 1–3, Coxsackievirus types A 9 and B 1–B 6, Echo 6 (strain D'Amori) and the two Echo 6 strains isolated from the patients. As seen in Table 1 no increase in neutralizing antibody titers against poliovirus 1–3 and Coxsackievirus A 9, B 1–B 6 was observed in both cases. Neutralization tests with the prototype Echo 6 and with the isolates demonstrated that the antibody titers in convalescent sera were from 4 to 16 times higher than in the acute sera.

## Discussion

Echovirus type 6 has been recognized as an etiological agent of sporadic and epidemic meningitis, polio-like illness, encephalitis, Guillain-Barré syndrome, exanthem, myalgia, diarrhea and acute respiratory illness. In this report virus isolation and serological studies have demonstrated Echo 6 virus infection in two children of about three years of age who were admitted with symptoms of A.C.A. This indicates that this virus may also induce an acute transient cerebellitis. It is also possible that, if more cases were virologically examined, other groups or types of viruses in addition to those already mentioned could be included in the etiology of this syndrome.

The cerebellar signs lasted about ten days in the first and 2–3 days in the second case. Furthermore, in the latter case an erythematous rash was also present. It is noteworthy that this child had been fed with Sabin type I, six days before admission and four days before the appearance of the rash. The hypothesis of a reaction to the Sabin strain [5] was ruled out both because polioviruses were not grown from rectal and pharyngeal swabs and because of the lack of antibody response against polioviruses. The presence of Echo 6 virus in the alimentary tract of the child probably inhibited the multiplication of the Sabin strain.

Considering the duration of the disease our second case who recovered after 3–4 days, should be regarded as a mild form of A.C.A. Most of the cases described as in our first patient, showed neurological abnormalities lasting 2–4 weeks after the onset of the symptoms [2, 3, 6, 16]; only in a few instances recovery took place only after one or more months [3, 13].

From an evaluation of the reports referring to etiological studies, it seems that

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CASE REPORT

# Congenital Nonhaemolytic Jaundice

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In 195., Crigler & Najjar reported seven related infants and children with "congenital nonhaemolytic jaundice with kernicterus". Since that time a few additional cases have been added to the world literature. The following is a report of a Swedish case, a child of a consanguineous marriage.

## Methods

Bilirubin determinations in plasma were performed according to Michaëlsson [7]. Pulmonary carbon monoxide excretion was studied according to Wranne [12]. Carbon monoxide in blood was also determined [4]. Otherwise standard laboratory methods were employed.

## Case Report

M. L., a boy born August 15th, 1961, the second of two children. The parents are normal and are first cousins (Fig. 1). The boy's great-grandfather had been very dark-skinned but otherwise apparently healthy and lived to the age of 70 years. The parents and the sister of the patient were not jaundiced and appeared healthy. The laboratory investigations of the parents are shown in Table 1. Among the other relatives there were no known cases of jaundice, neurological disorders or deaths in infancy. The boy's mother was healthy during pregnancy. H. was born  weeks after the expected date and his birth weight was 3450 g. Five mg menadione were given orally. Slight jaundice was observed by the mother on the second or third day but was considered insignificant. Mother and child were discharged from the obstetric department on the fifth day. However, the jaundice increased and, at the age of fourteen days, the boy was admitted to the Paediatric Department of the Uppsala University Hospital.

On admission, physical examination revealed nothing abnormal except jaundice. There were no signs of bilirubin encephalopathy. *Serological studies* showed that the blood group of both the mother and the infant was A Rh(+). Coombs direct and indirect tests were negative in both mother and child. *Haematological studies* are shown in Table 2 together with the results of *bilirubin determinations*. The urine contained no urobilin, urobilinogen or bilirubin. The faeces contained on some occasions traces of bilirubin but no urobilin or urobilinogen. The following tests of *liver function* revealed nothing abnormal: serum GOT, the thymol reaction, alkaline phosphatases, serum lipids and proteins and the prothrombin-proconvertin level. Laparotomy was performed when the boy was two months old. The liver appeared normal and an X-ray investigation of the biliary ducts at operation revealed no malformations. A biopsy of the liver was performed and the specimen kindly examined by Professor G. Hultquist.

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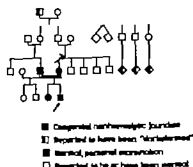



Fig. 1 Pedigree.

The results were inconclusive, however regarding the aetiology of the jaundice. The architecture of the liver parenchyma was normal and there were no signs of inflammation or degeneration. Only small amounts of bile pigments were seen.

Syphilis, toxoplasmosis, cytomegalic inclusion body disease, urinary tract infection, sepsis, hypothyroidism, galactosaemia, glycogenosis and pyknocytosis were excluded by appropriate tests. At the age of 2½ months the boy was still heavily jaundiced and plasma bilirubin determination revealed a concentration of 1 .5 mg/100 ml. There were still no signs of neurological involvement. The boy was discharged.

During the following years, his motor and mental development were normal. At eighteen months he was able to walk by himself and utter a few words. At 2 years, he spoke simple sentences. H was able to control his micturition from the age of two

TABLE 1 *Laboratory investigations on parents*

| | F ther | Mother |
|---|---|---|
| Haemoglobin g/100 ml | 14.8 | 12.6 |
| Reticulocytes % | 1.6 | 2.0 |
| Platelets per cu. mm | 210,000 | 104,000 |
| Leucocytes per cu. mm | 8,700 | 4,20( |
| Bilirubin mg100 ml | | |
| total | 0 9 | 0.8 |
| conjugated | 0 | 0 |

TABLE 2. *Haematological data of the patient*

| | Age | | |
|---|---|---|---|
| | 2 weeks | 2 months | $3\frac{4}{12}$ years |
| Haemoglobin g/100 ml | 17 4 | 10.6 | 11.6 |
| Reticulocytes % | 1 0 | 2.8 | 1 2[c] |
| Platelets per cu. mm | 195,000 | 189 000 | 280,000 |
| Leucocytes per cu. mm | 8,700 | 10,600 | 8,400 |
| Bilirubin mg/100 ml | | | |
| total | 24 0 | 19.6 | 11.1 18.0 |
| conjugated | <2 0 | < 2.0 | < 2 |
| Alkaline phosphatase[a] | 8.5 | 6.8 | 4.4 |
| Serum GOT units | — | 62 | 24 |
| Serum GPT | — | 22 | 7 |
| Haptoglobin mg/100 ml | — | 25 | 60 |
| Carbon monoxide excretion micro-liters per hour | — | — | 43 |
| Daily haemoglobin catabolism[b] gram | — | — | 0.8 |
| CO-Hb % | — | — | 0.4 |
| Hb F % | — | — | 0.2 |
| Hb $A_2$ % | — | — | 2.1 |
| Spherocytes | Not found | Not found | Not found |

[a] Bessey and Lowry units.
[b] Calculated from CO data.
[c] Mean of 10 determinations.

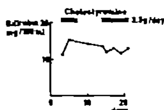


Fig. 2. Therapeutic trial with cholestyramine.

years. His ability to climb and jump did not indicate any disturbance of the equilibrium.

Aged 3 1/12 years, the boy was readmitted for follow up investigations. Except for jaundice physical examination showed nothing abnormal. His body weight was 13.5 kg and height 96 cm (mean value for Swedish children 14.8 kg and 97 cm). His teeth were not visibly discoloured. There were no signs of ataxia. Motor examination revealed that the "motor ages" of the upper and lower extremities were 43 and 4_ months, respectively. (The chronological age was 40 months.)

Audiometry and an electroencephalogram gave normal results. Haematological studies and bilirubin investigations are shown in Table 2. Starch slab electrophoresis of the haemoglobin revealed only normal fractions. The osmotic fragility of the red cells was within normal limits. There were no signs of disturbed liver function apart from the hyperbilirubinaemia.

Cholestyramine (kindly supplied by Merck, Sharp and Dome International) 3.3 g daily was given during a 4-day and a 10-day period. The therapeutic effect on the bilirubin concentration seemed negligible (Fig )

## Discussion

The present patient has a long standing hyperbilirubinaemia of the indirect type without other signs of disease. The two most probable explanations to this seem to be the hyperbilirubinaemia described by Arias [1] and the syndrome of "congenital familial nonhaemolytic jaundice with kernicterus" described first by Crigler & Najjar [4]. Arias' patients had bilirubinaemia of the unconjugated type the plasma concentrations varying between 0.4 and 19.9 mg/100 ml. In the majority of the patients jaundice was not detected until a year or longer after birth. Thus it seems less probable that the present patient has a disease of the Arias type. However his disease is well in agreement with the Crigler Najjar syndrome without neurological involvement. Similar cases are described by Childs & Najjar [3], Sugar [11] and Schmid & Hammaker [10].

### *Bilirubin metabolism*

When the present patient was 3 1/12 years old the total haemoglobin mass was calculated to be about 100 g. Assuming that



Fig. 3. Plasma bilirubin concentration in the patient compared to the haemoglobin catabolism of normal infants (0–100 days) and to that of the patient (3 1/12 years).

his erythrocyte survival was 1?0 days, his daily haemoglobin breakdown would be about 0.85 g. Carbon monoxide studies revealed a value around 0.8 g. Increased bilirubin formation from haemoglobin catabolism thus seems improbable. Billing et al. [ ], using $C^{14}$ bilirubin and $N^{15}$-glycine arrived at a similar conclusion concerning the haemoglobin breakdown in the Crigler-Najjar syndrome. Furthermore they found a normal production of the early-labelled pigment. They supposed that bilirubin was eliminated by some other process than conjugation.

During the first months of life of the present patient, the bilirubin concentration in plasma showed a steady decrease. Theoretically this could be explained by an increasing efficiency of the bilirubin elimination process. However it could also be secondary to a diminishing bilirubin formation. Since there is no evidence of an abnormal haemoglobin catabolism in the Crigler-Najjar syndrome the plasma bilirubin concentration in the present case was correlated to the haemoglobin catabolism of normal infants [13]. As can be seen in Fig. 3 a rather close correlation was found. The decrease of plasma bilirubin thus seems to be due to diminution of the bilirubin formation rather than to an increased efficiency of the bilirubin elimination.

*Danger of neurological damage*

Rosenthal et al. [8] described a boy who was presumed to have a Crigler-Najjar syndrome and who showed normal neurological development up to the age of three years. At this age slurring of the speech and an intention tremor were noted. After some weeks, improvement ensued but was not complete. Two years later the condition deteriorated. The boy was unable to walk, and showed a marked intention tremor and a slow and scanning speech. The plasma bilirubin concentration then was between ?0 and 35 mg/100 ml. The bilirubin level during his "normal" period was not known. No reason for the delayed deterioration of his neurological function could be given.

It is thus obvious that neurological damage from hyperbilirubinaemia can occur long after the neonatal period. The danger for the present patient is certainly not over yet. In the neonatal period, exchange transfusion is the treatment of choice. This can also be done without too much difficulty in a child of pre-school age but is somewhat cumbersome especially if a number of exchanges becomes necessary. Lester et al. [6] have suggested the use of Cholestyramine resin as a means of reducing unconjugated hyperbilirubinaemia. It was thought important to test this resin in the present case. No effect was found during a four day and a ten-day test, and this was as expected from the lack of effect in jaundiced premature infants [9].

## Summary

A boy with congenital nonhaemolytic jaundice is described. His parents were first cousins. There were no other known cases in the family. The patient was followed up to the age of 3½ years and showed no signs of neurological damage. Plasma bilirubin decreased from 24 to 12.8 mg/100 ml between the ages of 2 weeks and 3 months. A correlation was

found between this fall of bilirubin concentration and the haemoglobin catabolism in healthy infants. Thus it seems probable that the plasma bilirubin decreased because of decreased bilirubin formation and not because of increased efficiency of the bilirubin elimination.

A therapeutical trial with Cholestyramine resin did not reduce the plasma bilirubin.

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CASE REPORT

# Tietze s Syndrome in Childhood

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In 19*1 Tietze [6] described a condition of painful non-suppurative swelling of the costal cartilages. The syndrome has been recorded in both sexes with the highest incidence in young adults Tietze s patient was an 18-year-old male who had involvement of the second costal cartilage this costal cartilage is most often affected but multiple involvement also occurs. Chantraine [2] reported fourteen cases and suggested the name "chondropathia tuberosa" Kayser [3] reviewed 159 cases in the literature and reported another typical case occurring in a 16-year-old girl He found the age incidence in the 83 cases where the age was recorded to range from 11 to 79 years with mean age of 32.3 years.

The aetiology is not known and many possibilities have been discussed, in those cases where biopsies have been done no specific changes have been recorded. In the differential diagnosis of chest pain, this disease must be considered.

It is the purpose of this paper t record two cases of this disease occurring in children.

## Case Reports

R. D 3½ year-old boy had been noticed to have a cardiac murmur and also lump on the left side of the hest. H had previously had measles and chicken pox and there was nothing relevant in his family history He had been fully immunized and had normal milestones of development On clinical examination the positive findings were soft systolic murmur at the lower left sternal border which was considered t be functional and of no significance. H had smooth firm swellings of the eighth and ninth left costochondral junctions; these were tender and had normal overlying skin. N thing else abnormal was detected clinically. Investigations included a blood count sedimentation rat blood urea, urinalysis, E C.G and x-ray of chest and ribs, and all these were normal. H was seen at *intervals* for the next 15 months and the swellings although still apparent did not leave any sequel.

C J B., a 10-year-old schoolboy had a history of right upper abdominal pain which was associated with some anorexia and had been present sporadically for about 3 months. There was no pyrexia. H had only had measles previously and tonsillectomy 4 years before this illness. On linical examination the only positive observation was some tenderness in the right hypochondrium.

During the course of further bservation and after period f bout 4 weeks, painful swellings were noticed on the right side of the chest There was no history of trauma. These swellings were smooth and affected the right 3rd, 4th and 5th costochondral junctions; they were tender to palpation and had normal overlying skin. There was no fluctuat on Investigations included a blood

count, which was normal apart from a slight neutropenia and a normal E.S.R. x ray of chest and ribs and urinalysis were normal. No L.E. cells were seen and the Tuberculin test was negative. In the course of further observation similar tender swellings developed at the 6th and 7th left costochondral junctions. In the succeeding weeks the swellings became less tender and also slightly smaller. A year later the swellings had become very much smaller and there were no subjective symptoms relating to them.

## Discussion

Two cases of Tietze's syndrome are presented. Both were males and were younger than any mentioned by Kayser [3] in his extensive review of 160 cases in the literature where his youngest was 11 years of age. There is little doubt that the disease is rare in childhood but does occur and should be considered in patients with chest pain, careful palpation of the costal cartilages is necessary to diagnose the condition. The two patients recorded here had no obvious predisposing factors and no aetiological factors were discovered. It appears to be a self limited disease with no sequelae and there is no specific therapy available. Beck & Berkheiser [1] recorded four cases, one of whom was an 11 year-old male: the affected cartilage in these cases were all microscopically normal, and in these patients the swellings remained present for 3 to 5 years. No radiological abnormality has been reported and this was the case in the two patients recorded in this paper. Motulsky & Rohn [5] described two cases of this syndrome in patients with Hodgkins disease. Other specific diseases may also produce a similar clinical picture and these include benign tumours such as chondromata and pyogenic inflammatory lesions. Tuberculosis may also produce costal cartilage lesions and Maier [4] has recorded typhoid lesions affecting these areas. Rheumatoid arthritis may also affect the costochondral junctions.

## Summary

Two cases of Tietze's syndrome in childhood are reported and described. This condition must be considered in the differential diagnosis of chest pain and careful palpation of the costal cartilages is necessary to diagnose the disease.

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**CASE REPORT**

# Ovarian Changes in Ataxia Telangiectasia

by MICHAEL E. MILLER[1] and JANE CHATTEN[2]

*From the Children's Hospital of Philadelphia and the Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pa., U.S.A.*

Ataxia telangiectasia is a well recognized syndrome involving several body systems [1 5, 6] Recently attention has been drawn to the frequency of immunologic involvement in this disorder [10]. The purpose of this report is to describe the occurrence of ovarian follicular agenesis in a child with ataxia telangiectasia and to emphasize the frequency of ovarian abnormalities occurring in this syndrome

## Case Summary

D T an 11 year-old Negro female was first noted to have a difficulty walking at age 14 months. Previous growth and development were normal. She developed progressive "staggering" over the next year and continued to deteriorate neurologically. By seven years of age she was noted to have a widespread gait; poor balance; slow dysarthric speech; profound intention tremor; dyskinetic posturing; lateral nystagmus; and poor associated arm movements on walking Conjunctival telangiectases were first noted at this time and increased over succeeding years (Fig 1). At age 9, she began to develop upper respiratory infections with increasing frequency Summary of immunologic evaluation performed at this time revealed:

1 Inability to be sensitized to repeated applications of 2.4 DNFB.

2. Negative skin test with monilia antigen.

3. Markedly depressed response of peripheral lymphocytes to *in vitro* stimulation with phytohemagglutinin.

4. Absence of IgA on single ring diffusion with normal amounts of IgG and IgM.

5. Poor antibody response to diphtheria, tetanus and polio I antigens.

6. Normal inflammatory cycle as performed by the technique of Rebuck [13].

She developed pneumonia at age 10 and over the next year her course was characterized by repeated bouts of severe bacterial pneumonia, increase in severity of neurological abnormalities and in prominence of telangiectases Death occurred at age 11 following a severe respiratory infection

## Pathology

The body was that of an emaciated, prepubescent Negro female of normal stature

*Genitalia.* The uterus, ovaries and fallopian tubes were of infantile proportions but free of gross lesions. On histological examination, there was a striking paucity of follicular structures (Fig 2). Many semiserial cross-sections were examined, and each contained only one to four primordial

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Fig 1 Prominent telangiectasia of bulbar conjunctivae can be seen.

follicles widely separated by histologically normal stroma. There was no evidence of follicular maturation or degeneration.

*Other findings* Gross and microscopic changes in the thymus, lymph nodes, spleen, and other lymphoid structures and in the CNS were similar to those previously reported in this syndrome [1 6, 11]. The lungs were the site of extensive acute and chronic inflammatory disease which was considered to be the immediate cause of death.

## Discussion

The ovarian defect described here in a child with ataxia telangiectasia seems far more frequent than previously recognized In addition to our patient a previous report by Bowden *et al.* [*] described a similar abnormality of ovarian development occurring in association with ataxia telangiectasia and we are aware of two additional examples of the same association [3] Further Dunn *et al* [5] have described a child with ataxia telangiectasia found to have bilateral ovarian dysgerminomata This is considered to be a tumor of undifferentiated germ cells [4 8] Finally Boder & Sedgwick have described a female with ataxia telangiectasia showing no evidence of ovarian tissue on postmortem examination [1].

Thus, we can cite a number of cases of ataxia telangiectasia with associated abnormalities of primordial germ cell development. The true incidence of this association may be still higher if one were to carefully examine ovarian histology on all patients with ataxia telangiectasia. We are unaware of analogous testicular abnormalities occurring in association with ataxia telangiectasia. It is possible that the analogous testicular tumor seminoma [7] may be observed in the future

While one can only speculate regarding the etiologic significance of the high in



Fig. — (A) Section of normal ovary showing numerous follicles of varying type (B) Section of ovary from patient Note extreme paucity of recognizable follicular structures

cidence of ovarian abnormalities in this syndrome it is interesting to note that the germ cell is now regarded as deriving from yolk sac entoderm [15 14]. The embryonic cells are transferred from the yolk sac to the hind gut where they ascend through the mesentery toward the mesonephric folds [12 16]. During this migration the germ cells are intimately in contact with mesenchyme so that mesenchymal involvement [11] in ataxia telangiectasia could be associated with abnormal development of the germ cell

Homozygous $W_j$ strain mice show an identical germ cell ovarian abnormality [9]. The possibility of a relationship between retardation of travel through mesenchyme and the developmental germ cell defect in these animals has been raised [9]

It is hoped that recognition of the high association of ovarian abnormalities with ataxia telangiectasia will prove of value in future investigations involving the pathogenesis of this syndrome

## Summary


The occurrence of ovarian follicular agenesis in association with ataxia telangiectasia is described. The high frequency of this association is emphasized. Speculation is made regarding the significance of this association.


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# The Danish Pediatric Society

## Meeting March 9 1966

*B Friis-Hansen* Demonstration of a girl aged five years with xanthomatosis

*P Plum*. Report of the plans made by the medical research committee (LFK) for registration of current medical research

*Niels Hjorth and Kristian Thomsen:* Pustular parakeratosis (91 cases of a characteristic skin condition in children)

DISCUSSION

*J Kringelbach* inquired how frequently pustules were present.

*Hjorth* In pproximately a fourth of the cases.

*B Friis Hansen*. What is the incidence of the condition?

*Hjorth* Twenty cases were collected in Rødovre (western district f Copenhagen) in the course of three years. The condition is *considerably rarer than Besnier s prurigo* but much more common than mycoses in this age group.

To a question bout treatment from *E Wamberg* and *E Thamdrup, Hjorth* replied that local therapy with steroids and ant biotics resulted in clinical freedom from symptoms in the course of 3–4 months.

*El n Fog* mentioned the differential di agnosis from ichth oses.

*Niels Hjorth H K pp and P Osmundsen.* Cian ttl-Crosti's Disease

(Published in *Ugeskr Læg 1 5* 1335, 1966)

This skin condition, which is seen particularly in children under the ge f ix years, was illustrated by 122 cases from the western districts of Copenhagen. The main symptom is eruptions of rounded or possibly pointed papules of 1–3 mm which are bright red in the initial stages. The surroundings are initially red and warm and moderate itching is present The forearms and thighs are most frequently affected but all regions may be involved. The condition lasts for approximately six weeks. The etiology is unknown but the present material suggested an external mechanical or chemical cause The differential diagnosis from Besnier s prurigo is important For practical reasons, the above nomenclature was chosen for the condition.

DISCUSSION

*C Fridenchsen* recapitulated his previously published case (*Ugeskr Læg 1 5* 125, 1963) and discussed the nomenclature of the disease

*K Wilken Jensen* compared the condition with prickly heat

*Minna Yssing Herluf Jensen and Stig Jarnum.* Dietetic treatment f protein-losing enteropathy in inf cy

(Published in *Acta Paediat Scand* 56 172, 1967)

It proved difficult to carry o t the treatment on account of secondary dev lopment of allergy to casein for which reason the diet had to be commenced with skimmed breast milk to which 3½ % mid-chain triglyceride oil was added. S bsequently hypoweaning with skimmed cows milk was successfully carried out and this replaced breast

milk at the age of nine months and the diet was supplemented with rice-flour vegetables, lean fish, iron and vitamins. The daily ration of naturally-occurring fat in this diet did not exceed 2.5 g. The patient has been followed-up until the age of 11 months. The long-term prognosis is unknown as none of the patients previously reported have been followed-up for more than two years.

DISCUSSION

*P. A. Krasnilall ff* mentioned protein metabolism in normal infants as revealed by investigations with human albumin marked with [131]I and found that the patient reported above was at the upper limit of normal.

*B. Friis Hansen* inquired whether the serum lipoid values had been investigated.

*M. Young*: Yes, the values were normal.

*Herluf Jensen* stated that the synthetic oil was an American preparation.

*Niels Hjorth and H. Kopp*: Hand Foot Mouth Disease

(Published in *Ugeskr Læg. 128*: 293, 1966)

This condition has previously only been described abroad. The main symptom is eruptions of blisters on the hands, feet and/or in the mouth. After 3–5 days, the blisters dry up lea ving brown patches and the disease disappears in the course of 5–10 days without treatment. The condition is due to the Coxsackie virus type A 16.

## Meeting April 20, 1966

*K. Vollmond*: Demonstration of a girl aged four years with a mediastinal tumour due to von Recklinghausen neurofibromatosis.

*Olaf Andersen*. Lead poisoning

The Danish National Health Service has become aware of the increasing frequency of lead poisoning, particularly in U.S.A. In New York, lead poisoning is the cause of 0% of the fatal cases of poisoning. According to this, 10–20 cases of lead poisoning should be anticipated annually in Copenhagen. The Department of Occupational Health is planning an investigation in Denmark. The various manifestations of lead poisoning should be borne in mind, e.g. abdominal colic in children and particularly children with pica.

DISCUSSION

*G. Englesen* (Lund): W ha seen cases of lead poisoning in children which occurred after eating lead weights from curtains.

*B. Zachau-Christiansen*: An increasing number of cases of lead poisoning has also been reported in Britain.

*Th. Rasmdal and Ib Boesen*. Cardiothoracic index and heart volume in infants with congenital heart disease

The size of the heart expressed by the cardio-thoracic ratio

$$(CR) = \frac{\text{Transverse diameter of the heart}}{\text{Maximum internal diameter of thorax}}$$

and the volume of the heart ($\frac{1}{2}\pi$ length breadth diameter (correction for increase in the diameter of the heart on radiographic examination in the sitting position with focus/film distance of 147 cm is included) was calculated in 559 infants under the age of 12 months with verified congenital heart disease of 30 different types. The upper limit for CR was 0.65 and for the cardiac volume per m² was 300 ml.

| | | |
|---|---|---|
| Cr in 559 infant | < 0.65 | > 0.65 |
| | 80% | 20% |
| Cardiac volum (ml/m²) in 473 infants | | |
| | < 300 ml | 300 ml |
| | 25% | 75% |

If assessment of the size of the heart based upon CR with the limit 0.65, the

heart is only enlarged in ⅓ of 539 infants with congenital heart disease and in ⅔ the size of the heart is within normal limits. In contrast to this, the cardiac volume per m in 473 infants was over the upper limit of normal (300 ml) in ⅔ of the material and was under this limit in only ⅓. If the limit for CR is put as low as 0.50 eight infant out of 41 were found with heart volumes of more than 300 ml. Only when the CR is over or equal to 0 65, does this indicate enlargement of the heart as only three out of 94 infants in this group had heart volumes per m of under 300 ml.

Calculation f the heart volume per m is thus necessary in order to differentiate between an abnormally enlarged heart and a heart of normal size and the investigation confirms the previous conception that CR cannot be employed for this purpose

DISCUSSION

*Ol J Andersen* Were infant with indices of under 0 60 not submitted to further investigation?

*Ib Boesen* Yes, slightly increased indices must be controlled

*P Plum* What is the normal cardiac volume in normal neonates?

*Ib Boesen* This is not known but according to the graphs available it may be estimated to approximately 50 ml.

*B Friis Hansen*. How many infant with CR of over 0 60 are normal?

*Ib Boesen* In this material, five per cent

*J Vesterdal* emphasized the importance of taking the radiographs under the correct conditions.

*B F is Hansen* stressed that Du Bois formula is based upon a very limited material which did not include children.

*Hans T Lund* Congenital nephrotic syndrome

(Published in *Nord Med* 216, 1967)

A total of 57 cases of the congenit l nephrotic syndrome has been collected from the literature. Twenty-eight of the children were born prematurely and 20 cases occurred among siblings. Consanguinity is reported in isolated cases but the syndrome has not been described in more than one generation.

The majority of children die during the first year of life Only three of the cases reported ha e survi ed for more than five years. In several cases anti-renal and anti-placental antibodies were found both in the serum and bound to the renal tissue suggesting a foetal immunological process.

I have examined a boy and a girl with the syndrome where the course was strikingly prolonged. Neither consanguinity nor familial predisposition were present. The pregnancies and deli eries were normal. The girl was born four weeks prematurely and the birth weight was 2400 g Both patients developed nephrotic syndromes at the age of ½ months and in the girl, this was preceded by a staphylococcal infection of the scalp. Renal biopsies in both of the patients revealed slight glomerulitic changes which showed progression in the boy in repeated biopsy specimens. Investigation for antirenal antibodies was negative in both patients. During the past year the oedema has disappeared The general conditions nd the renal functions are good but the proteinuria is still massi e Particularly in the girl the height and weight are considerably less than the normal for the age

DISCUSSION

*G Engleson* (Lund) inquired whether thymectomy had been ttempted

To a question by *B Zacho -Christiansen* about the method of n estigation for antirenal factors, it was stated that this w performed by indirect fluorescent microscopy

*H ing Andersen* reported that in addition to Finland, the condition is common in Minneapolis It is possible that the st phylococcal infection in one of the patient ini tiated the process.

*C J Ingemar and E. Tersler* Examination of duod nal j ices

I 112 fant (4 normal infant and 70 infants in whom malabsorption was suspec

ted on clinical grounds) the content of amylase, trypsin and lipase in the duodenal juice was investigated. The investigation wa un dertaken either on fasting duodenal juice or following stimulation with 10% Eledon[4] and subsequent withdrawal over a period of one hour Employing both methods, con siderable variations in the quantities of all three enzymes were found both in normal and in sick children and neither of the methods presented specific advantages over the other With several other authors, we found low amylase values in infants under the age of six months.

Definitely lowered values for all three en zymes were encountered by both methods only in children with cystic fibrosis. In the remaining children, by and large enzyme activity was found which did not deviate from that in normal children. A group of children with chronic diarrhoea, steator rhoea, normal xylose tolerance tests and normal sweat tests showed low enzyme values in isolated cases and it is concluded that examination of the duodenal juice is indicated particularly in children with chronic diarrhoea of this type

DISCUSSION

*O Hansted*. Some of the cases mentioned resemble patients described by Sheldon and by Dorothy Andersen.

*O Mellerioee* (Lund): In cysti fibrosis, increased iron absorption is encountered, i.e. not anaemia. This is in accordance with the findings in the present material.

*Henning Andersen* mentioned correspond ing investigations which he had performed some years ago which re ealed, inter alia, low amylase values under the age f 3–4 months. H discussed the method of investi gation and recommended the use of a double catheter with the possibility of withdrawing gastric juice.

*S Mullertz* accounted for the methods of investigation employed and participated in continued discussion of which method could be considered most suitable.

*Gudrun Dahl and Ib Boesen*. Deafness and persistent ductus arteriosus

DISCUSSION

*Henning Andersen* inquired whether radiographic examination of the middle ears of the patients had been undertaken and whether any of the mothers had received sul phonamides during pregnancy

*Gudrun Dahl*. Neither radiographic exami nation nor investigation of maternal medi cation had been undertaken.

## Meeting June 15 1966

*K. Sierbæk Nielsen and J Melholm Hansen*. Thyroid function and plasma tyrosine in the neonatal period

(Published in *Acta Paediat Scand*, *56* 141 1967)

DISCUSSION

*B Friis-Hansen* inquired whether any relationship could be demonstrated between thyroid function and the serum bilirubin concentration. *K Sierbæk Nielsen* replied that there was no connection.

*E. B Buch, Henning Pedersen, B Zachau-Christiansen and C. Zimmermann-Nielsen*. Tyrosinosis

A case of tyrosinosis in female infant of four months is presented. The patient died six weeks after admission. No metabolic defect could be demonstrated in either of the parents, who were unrelated, or in a three year-old brother

Two forms of tyrosinosis exist One form presents during the first months of life with enlargement of the liver ascites, diffuse

amino ... ura, glucosuria and hyperphosphat ... and rickets and, as a rule ends fatal ... in the first year of life In the other form which commences during the first year of lif ... the condition is dominated to a greater extent by rickety skeletal deformities and impaired liver function, the course is much more prolonged and may be up to 20 years. Nodular degeneration of a cirrhotic nature is encountered in the liver degeneration of the proximal convoluted tubules, hypertrophy of the islets of Langerhans and rickety skeletal changes.

Investigation of the plasma concentrations of free amino acids, in particular tyrosine phenylalanine and methionine which are raised, is of special significance in the diagnosis.

Good results obtained elsewhere by treatment with diet low in tyrosine and phenylalanine and large doses of vitamin D were mentioned.

DISCUSSION

*O Engleson* and *B Palmgren* (Lund) mentioned two cases from the Children Clinic in Lund. A positive ketostix reaction led to the diagnosis in one of the patients. Treatment with a diet low in tyrosine and phenylalanine in one of the patients resulted in a definit effect with reduction of the tyrosinaemia and disappearance of the rickets.

*K Sierbæk Nielsen* asked whether treatment with cortisone had been attempted as this should lower the tyrosine concentration. *E B Buch* replied that Halvorsen had obtained poor results from cortisone therapy

*H Dyggve* asked whether mental retardation had been described in the disease to which *E B Buch* replied that it had not been described.

*O Strincke* Result f admissions to the Christmas Seal Home

(Published in *Ugesk. Læg* *1 I* 1102 1966)

In the period 1938–1963 1166 boys aged 6–14 years were admitted to the Christmas Seal Home ("Julemærkehjem") "Lindersvold" (Zealand) on account of psychic symptoms. The duration of the periods of admission was pproximately three months. Treatment included occupational therapy with pedagogic and individual advice but no actual psychological or psychiatric therapy Four hundred nd forty-six cases were illustrated as regards symptoms, environment and the immediate result of the st y in the home Attempts were made to follow up 360 of the patient and, in 283 cases, it proved possible to obtain personal contact with the parents. Follow-up contact was obt ined t least one year fter the stay in 74 per cent of the cases.

At follow-up examination, 37% were entirely symptomfree 19% partially symptomfree while 11% had shown improvement for only 3–6 months and the remainder had shown n improvement or only transient improvement

Taking into consideration the very serious psychic symptoms which the great majority of the boys had on admission, it is concluded that as a whole the stay in the home must be regarded as satisfactory and, for a great proportion of the children, as extremely f vourable

DISCUSSION

*E Thamdrup* The results reported must by and large be regarded as xcellent Any further treatment of these children fter the stay in the home must be the task of the departments originally referring them at follow-up control of the children. The Christmas Seal Home should scarcely be used as n institution for actual treatment but the existing home can be improved regards staffing and premises w th the fund available.

*P W Brandstrup.* I am lso of the opinion that the Christmas Seal Home should continue according to the present lines. Treatment must be considered to be cheap compared with treatment in other inst tutions. Efforts should be made however t provide further treatment for the children for whom a stay in the Christmas Seal Home proves insufficient

*J Kringelbach* Homes such these are

of great significance for paediatric departments all over the country as many patients will benefit from a period of convalescence after discharge from hospital.

*B Zachau-Christiansen.* Some of the funds obtained from the sale of Christmas Seals should be employed to defray the expenses of follow-up control of the children after a stay in the Christmas Seal Home

*Torben Iversen*

## ERRATUM

In the article by L. Victorin, An Epidemic in Newborns due to Infection with *Pseudomonas Aeruginosa*" vol. 56 no 4 the second sentence under 'Conclusion" page 347 should be changed as follows. "If performed it should take place in a bassinet cleaned with an antibacterial agent that is thoroughly washed away before the bath."

## BOOK REVIEWS

F Linneweh (ed ): Fortschritte der Pädologie

Vol. I. Springer Verlag, Berlin, Heidelberg and New York, 1965. 186 pp., ill. DM 49

According to the editor this volume is the first in a series t appear at regular intervals with the purpose of delineating the research frontiers in pediatrics and related fields of medicine. In this first volume the editor has gathered an international list of contributors. The book is written in German except for the last chapter which is in French. After an introductory paragraph by the editor on the postnatal adaptation with particular reference t enzyme function, the four following hapters are devoted t various aspects of protein metabolism Schreier and Porath, Heidelberg, report on the synthesis f protein in the pre- and postnatal period as studied in animals and v. Muralt from Bern reviews the same topic studies in the fetus and newly born infant. Oehme of Marburg discusses the intriguing phenomena associated with the concept of immunological tolerance and Haupt from Essen the clotting mechanism with particular reference to the neonatal period. Stave of Yellow Springs discusses the enzymatic activities in various organs in animals from intrauterine t adult life focusing on the glucolytic enzymes. In the next chapter H ber and Scheibl of Vienna report on the development of the fat resorption capacity in children during the first 6 months of life. Bruck of Marburg has written a chapter on the thermoregulation of the newborn infant and Knorr from Munich presents normal 24-hours values for the excretion of steroids in children from birth t young adulthood. The following chapters

are devoted to the placental gas exchange and to the gas transport capacity of the growing child. These are written by Bartels and Wulf and Riegel respectively all from Tubingen. In the last paragraph of the book M. Lelong and C. J Dalloz give a review in French of the information presently available regarding the regulation of muscular tone in the first year of life. As a source bibliography the volume, in its present form, should be a valuable asset, but from the standpoint of readily available information it is not always satisfactory It suffers from the point of view of brevity which makes the reading difficult. In the reviewer s opinion the book represents an ambitious attempt to review various current frontiers of pediatric research. A critical evaluation of the scientific information presented and the numerous reference sources is, however an almost impossible task for a single reviewer

*Göran Wallgren*

M *Cornblath* and *R. Schwartz*. Disorders of Carbohydrate Metabolism in Infancy

Vol. III in the series: Major problems in clinical pediatrics. Saunders, Philadelphia and London 1966. 297 pp., ill. £2 19s 6d.

In this monograph the authors provide the pediatrician an excellent summing-up of the current concepts of the normal and abnormal physiology of carbohydrate metabolism in the fetus, the pregnant mother the neonate and the infant. The book consists of twelve chapters the first three of which give the basal information of the metabolism of carbohydrate, the metabolic adjustments in pregnancy and the carbohydrate homeostasis in the neonate. The other nine chapters deal with problems f the newborn and neonate hereditary metabolic disorders, hypoglycemic syndromes in infancy and sugar malabsorption syndromes. Each chapter is followed by an extensive bibliography Diets for disorders of carbohydrate metabolism and a list of carbohydrate content of food are given in two appendicis. Among many important subjects in this book the neonatal hypoglycemia might be mentioned. The fact that hypoglycemia of marked degree can be present without symptoms in the newborn infant has often led to the false conclusion that there is no relationship between hypoglycemia and abnormal signs. A valuable attention is paid to the diagnosis and the management of the clinical problems in this field. This book contains a considerable amount of information about an expanding section of pediatrics and is warmly recommended.

*O Hansson*



# Sture Siwe †

Sture Siwe who died in April 1966 was born in Karlskrona in 1897 He matriculated in Karlskrona in 1914 and then came to Lund, and years later took an intermediate degree (fil. kand.) in theoretical and practical philosophy pedagogics and history of religion. It is quite conceivable that he had contemplated becoming a clergyman. But he switched over t medicine and took his diploma in 19*4 and wrote a doctoral thesis in 1926. In 19*7 he was appointed reader in anatomy

As early as 1927 Siwe started his paediatric training at the Children's Hospital, Lund, under Professor Kjell Otto af Klercker. From 1934 to 1937 he was assistant chief paediatrician at the Children's Hospital, Lund. Finally in 1930 he was appointed Professor of Paediatrics after hard competition.

Siwe's thesis was entitled "Pankreasstudien" and may be regarded as a comprehensive comparative embryological study of the pancreas in vertebrates. The main conclusion of his thesis was that the acinar cells and the island cells are of independent genesis. Later Siwe published a case of exogenous pancreatic agenesis and regarded this as support for his conception. The patient was a child, almost 2 years old, with distinct clinical signs of exocrine hypofunction of the pancreas. In the careful post mortem histological examination Siwe showed the almost complete agenesis of the exocrine apparatus of the gland, while Langerhans islands showed no changes in appearance or number. This was the first case published of this type.

It is obvious that Siwe's training in anatomy and histology stood him in good stead in his clinical research. The first example of this was his paper in a case with a disease which still carries his name: "Die Reticuloendotheliose—ein neues Krankheitsbild unter den Hepatosplenomegalien". From this and published cases with similar changes Siwe recognized a well defined clinical entity which, though of obscure aetiology, must be distinguished from ordinary sepsis with secondary reaction of the reticulo-endothelial system. Later the disease was given the name of the two authors by whom it had originally been recognized, namely Letterer-Siwe's disease. That paper did not at that time attract the interest it deserved. It passed more or less unnoticed until later when Arvid Wallgren found the clinical symptoms to fit into a picture of histiocytosis in the broad sense of the term, a clinical entity embracing not only Letterer-Siwe's disease but also Morbus Hand-Schüller-Christian and eosinophilic granuloma. On introduction of the term histiocytosis so-called transitional cases played an important role, i.e. cases showing features of more than one of the three conditions. Siwe, however, refused to accept the occurrence of such cases.

Siwe was also interested in the problem of the pathogenesis of spasmophilia. Using an apparatus for microtitration and an electromagnetic stirrer described by Linderström-Lang-Holter, Siwe succeeded in modifying a previous method for determination of the blood calcium according to Kramer-Tisdal respectively de Ward so that the determination could be made in such small amounts of the capillary blood as 0.2–0.3 mm³. This enabled daily measurement of the calcium at short intervals. Siwe made determinations on spasmophilic children at such brief intervals as 30 minutes and found a fundamental difference between normal and spasmophilic children. In the former the calcium level was practically constant, while in the latter it fluctuated considerably. Even though the calcium level was on the average depressed, a sudden fall in the level was characteristic of children with spasmophilia. This lability of the calcium level has since been confirmed by other researchers.

In some other investigations Siwe stud

led the mechanism of the parathyroid regulation of the blood calcium-phosphorous level a problem which was, and still is, of central paediatric interest in the question whether spasmophilia is associated with a disturbance of parathyroid function. Opinions on this point have varied from time to time. When Siwe began his investigations it was believed that a high blood phosphorous level after removal of the parathyroid argued against the assumption that spasmophilia has anything to do with parathyroid insufficiency Siwe was able to show—both in animals and in patients who had undergone parathyroidectomy in association with operation because of goitre—that a high serum phosphorous level was not obligatory in hypoparathyroidism and thus that the absence of increased serum phosphorous in spasmophilia did not argue against a relationship with parathyroid insufficiency Siwe's assumption that impaired parathyroid activity contributed to the low serum calcium in spasmophilia has later gained new support.

Siwe was interested in questions concerning behaviour disorders in children and childhood delinquency but he doubted whether it was wise to set up child psychiatry as a separate discipline and claimed that every paediatrician should also be a child psychiatrist. He also found time for the social welfare of children.

Siwe was no great friend of paragraphs, restrictions and regulations. His criticism was leveled at bureaucracy and often coloured by his local patriotism. He was not always able to accept certain formal requirements of society and as he grew older he left the impression of a dreamer and philosopher He seemed to be finding his way back to the first years at Lund when, as mentioned above he came to study philosophy and history of religion.

*Bertil Lindquist*

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# Immunoglobulin Levels in Healthy Children

by S GUNNAR O JOHANSSON and TORSTEN BERG

A knowledge of the immunoglobulin levels in healthy children of different ages is necessary when estimating immunological deficiency states of various kinds In the course of the last few years the concentrations of the immunoglobulins IgG IgA and IgM in healthy children have been published in several reviews [3 6 11 1*]

The reported normal serum levels have shown considerable differences from author to author This may be due to the choice of methods used, to the selection of children and probably also to real differences between different populations. To our knowledge no systematic study of IgD levels in children has been published.

We report here the levels of the immunoglobulins G A M, and D in healthy children from the newborn up to five years of age The results are compared with the immunoglobulin levels in adults. One of the purposes of this investigation has been to establish the lower normal levels of the immunoglobulins in a population of healthy children.

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## Material and Methods

Umbilical cord blood from 16 full term newborns, and capillary blood from 172 healthy children with ages ranging from 6 weeks to 5 years have been examined. Equal numbers of males and females were studied. Age distribution within the groups is shown in Table 1 The samples were collected at a Baby Welfare Centre in Uppsala mainly during the spring and autumn of 1966; a few samples were obtained during the first months of 1967 Children with frequent infections even if moderate or slight of the upper respiratory tract and those with a history of any severe infection or with allergic symptoms have been excluded. Children with slight infections of the upper respiratory tract have been included if they had been free of symptoms for at least a fortnight before sampling With few exceptions the children had been vaccinated according to the ordinary scheme i.e. B.C.G small pox, pertussis, tetanus, diphtheria and poliomyelitis.

About 0.5 ml of capillary blood was obtained from the heel or finger tip and after clotting and centrifugation the sera were kept at –20°C until analyzed. Quantitative determinations of the different immunoglobulins were made by single radial immunodiffusion in agar gel using a modification [7] of the method of Mancini *et al.* [9]. This method has a good precision, s D 5 8 per cent and a fairly high sensitivity the lower limit of detection being about 1 mg per 100 ml of the respective immunoglobulin. For

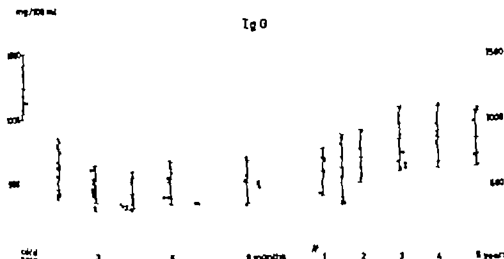



Fig. 1 The concentration in mg/100 ml of immunoglobulin G in each individual compared to mean values ±1 S.D

comparison immunoglobulin levels were determined in serum samples from 97 healthy individuals between 20 and 70 years of age selected at random from health examination in the county of Uppland [7].

## Results

The immunoglobulin levels in healthy Swedish children up to five years of age are shown in Figs 1–4 and a summary and comparison of mean values in children and adults is given in Fig 5 and Table 1

The IgG level of cord serum was 1240 ± 259 (1 S D) mg per 100 ml. At six weeks of age the value had dropped to 617 ± 104 mg per 100 ml and showed a minimum at 4½ months (428 ± 114). The

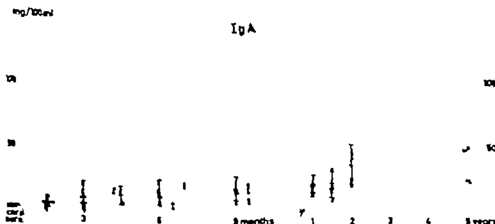



Fig. — The concentration in mg/100 ml of immunoglobulin A in each individual compared to mean values ±1 S.D

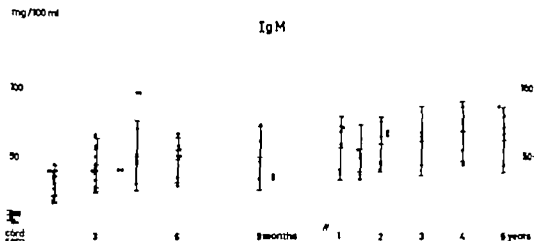



Fig. 3. The concentration in mg/100 ml of immunoglobulin M in each individual compared to mean values ±1 S.D.

level remained remarkably low (about 500 mg per 100 ml) during the first year and at the age of 1 year a mean value of 500 ± 183 mg per 100 ml was found, i.e. scarcely half of adult level. The level of the adult (1323 ± 279 mg per 100 ml) had been reached at 5 years of age the concentration being 840 ± 218 mg per 100 ml.

IgA was not detectable at birth but was found in all children at 6 weeks of age with a mean level of 6.9 ± 4.6 mg per 100 ml. Then the IgA level rose slowly up to 21.2 ± 8.2 mg per 100 ml at 1 year of age

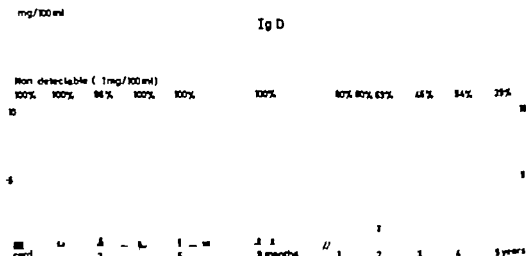



Fig. 4. IgD concentration in mg/100 ml in each individual. The frequency of individuals lacking IgD (IgD < 1 mg/100 ml) is given for each age group.

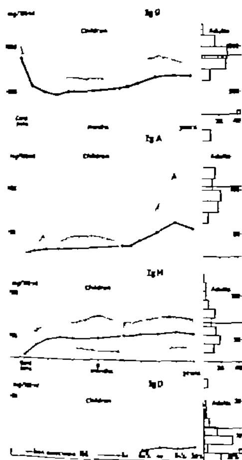



Fig. 5. Average levels and range for the immunoglobulins in 158 healthy children up to 5 years of age compared to immunoglobulin concentrations in adults given as frequency diagram.

which is 13 % of the adult level (158 ± 60.5 mg per 100 ml). At 4–5 years of age, the level was 69.5 ± 35.6 mg per 100 ml which is about 40 % of adult level.

The IgM concentration at birth was 7 ± 2.6 mg per 100 ml. A rather rapid rise in the concentration was seen during the first weeks and about half of the adult level (88.4 ± 43.4 mg per 100 ml) was reached by 3 months of age (45.3 ± 17.6 mg per 100 ml) the concentration at 4–5

TABLE 1 *Immunoglobulin levels in mg per 100 ml in 158 healthy children compared to adults*

| | | IgG | | | IgA | | | IgM | | | IgD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Mean | s. | Range | Mean | s. | Range | Mean | s. D | Range | Mean | s. D | Range |
| Cord serum | 15 | 1210 | 250 | 837–1723 | 1 | | <1 | 7.7 | 3.6 | 3.2–11.0 | <1 | | <1 |
| 6 weeks | 21 | 617 | 164 | 330–858 | 6.9 | 4.6 | 0.9–21.7 | 31.8 | 13.5 | 13.3–71.0 | <1 | | <1 |
| 3 months | 34 | 193 | 148 | 257–921 | 11.9 | 11.8 | 2.0–57.1 | 45.3 | 17.6 | 18.0–80.5 | <1 | | 1 3.3 |
| 4½ months | 19 | 128 | 114 | 289–666 | 12.5 | 7.0 | 5.1–30.0 | 50.5 | 5.0 | 19.8–94.8 | 1 | | 1 |
| 6 months | 20 | 509 | 167 | 220–816 | 15.3 | 9.9 | 2.5–17.0 | 47.9 | 10.1 | 27.2–90.8 | 1 | | 1 |
| 9 months | 17 | 511 | 103 | 330–701 | 18.2 | 9.3 | 6.9–17.0 | 49.8 | 23.7 | 18.0–101 | 1 | | 1 |
| 1 year | 10 | 500 | 183 | 321–850 | 31.3 | 8.3 | 6.9–34.0 | 54 | 23.3 | 20.3–91.5 | 0.7 | 0.8 | 1 1.9 |
| 1½ year | 10 | 6.1 | 57 | 336–1062 | 21.5 | 10.3 | 9.3–41.0 | 55.9 | 17.4 | 34.0–80.9 | 0.9 | 0.8 | 1 1.8 |
| 2 years | 13 | 705 | 109 | 453–1165 | 30.8 | 15.8 | 13.3–66.5 | 50.6 | 19.9 | 35.5–90.8 | 1.1 | 0.8 | <1 3 |
| 3 years | 11 | 813 | 241 | 613–1317 | 55.0 | 31.3 | 11.5–116 | 62.1 | 25.0 | 23.0–103 | 2.8 | 2.5 | <1 9.0 |
| 4 years | 13 | 853 | 233 | 380–1203 | 70.9 | 35.6 | 1.3–180 | 67.9 | 19.8 | 24.5–101 | 1.9 | 1.3 | <1 6.6 |
| 5 years | 13 | 810 | 218 | 500–1128 | 62.5 | 31.9 | 7.1–187 | 63.3 | 23.0 | 32.3–101 | 2.1 | 1.5 | <1–6.1 |
| Adults | 89 | 1223 | 279 | 756–2310 | 158 | 60.5 | 50.6–337 | 88.4 | 43.1 | 20.6–379 | 11.7 | 10.1 | <1–55.7 |

years of age was 66.1 ± 22.8 mg per 100 ml.

With one exception no IgD could be detected (sensitivity of the method 1 mg per 100 ml) up to 1 year of age. Then the number of children in which IgD was demonstrated increased to 61 per cent by 5 years of age. The mean concentration in all samples (amounts less than 1 mg per 100 ml calculated as 0.5 mg/100 ml) at that age was 2.4 ± 1.8 mg per 100 ml.

## Discussion

In assessing suspected immunological deficiency conditions clinically it may be difficult—especially with young infants—to evaluate the results of immunoglobulin determinations. A primary condition is to have a sufficiently sensitive and accurate method, another condition being to know the normal variations of immunoglobulin levels at different ages with the method used. The diagnosis of hypogammaglobulinemia cannot always be based on isolated results and the different immunoglobulins should be followed up with repeated determinations.

The children included in this study were selected to represent a healthy population. The type of selection used may result in a mean immunoglobulin level being lower than in a sample which includes healthy children whose immunoglobulin production has been stimulated by repeated low level infections. We have, however, been mainly interested in the lower limits of the respective immunoglobulins in healthy individuals.

As a matter of fact we have found remarkably low IgG values in our study especially during the first year of life. As can be seen from Figs. 1 and 5 an IgG level of 220 mg per 100 ml of serum in a nine-month-old infant is compatible with complete health as well as a level of 300 mg per 100 ml in a 4-year-old girl.

One factor that considerably influences the results of the immunoglobulin determinations is the choice of the immunoglobulin standard used. These methodological questions have been discussed elsewhere [7]. With the method used in the present study the results of the immunoglobulin levels in an adult Swedish population are in good agreement with results obtained by several other authors [2, 13]. The low concentrations of IgG during the first year of life thus do not seem to be due to a systematic technical terror. At one year of age the IgG level was approximately half of the adult level whereas Fulginiti *et al.* [6] found a level at one year of age that almost reached ¾ of the adult level. The results of Stiehm & Fudenberg [11] cannot be directly compared with ours as they have been given for different age samples.

IgA was found in only one sample of cord sera out of 16. This child, who had a concentration of 0.6 mg per 100 ml of IgA also had a strikingly high IgM value in the cord serum (33 mg per 100 ml). The child was delivered in a normal way and showed no signs of disease while staying at the maternity ward. The possibility of a transplacental transfusion has been discussed but not proved and this patient was excluded from the study. West *et al.* [1*] could not demonstrate IgA at birth or before the age of 25 days. Fulginiti and co-workers [6] demonstrated IgA in one child out of 18 at birth whereas Stiehm & Fudenberg found IgA in small quanti-

ties in ½ of new born infants and in all children above the age of 3 weeks. The finding of IgM in small quantities in all cord sera agrees well with results previously reported [5 11]. The rapid rise of the IgM concentration during the first weeks of life and the very slow increase of the IgA level is also in good agreement with previous findings [3 6 11 12].

The fact that the IgM level in normal individuals increases so early makes this immunoglobulin particularly suitable to follow when immunoglobulin deficiency is suspected in the newborn. However it has been possible to demonstrate synthesis of IgM in isolated cases of the immunodeficiency syndrome with the combination of lymphopenia and agammaglobulinemia [1 4]. Determination of the other immunoglobulins will probably not give any information to help the diagnosis of agammaglobulinemia at this age.

Fairly recently the fourth immunoglobulin class IgD was described by Rowe & Fahey [10]. It is a 7 S immunoglobulin, with an estimated molecular weight of about 160,000 which is said not to pass the placental barrier. Very little is known about its biological function, and to our knowledge no systematic study of its appearance in childhood has been reported. Rowe & Fahey [10] examined a few healthy children of the ages of 2–11 years and could not demonstrate a significantly lower level of IgD in those children compared to adults. In the present study the IgD level of 4–5-year-old children was only 20 per cent of the adult level. Rowe & Fahey did not find IgD in a couple of 4-month-old infants which is in agreement with our results.

In our study a 3-month-old infant had a concentration of 2.3 mg per 100 ml of IgD. This child had the highest levels of IgG (994 mg per 100 ml) and IgA (57 mg per 100 ml) and among the highest of IgM (74 mg per 100 ml) in its age-group. On examination the child showed no signs of illness and did not have any increased tendency towards infections. This child was not included in the summarizing Fig 5.

Twenty per cent of children aged 1 year had detectable levels of IgD. The percentage of children with detectable IgD as well as the mean values of the concentration then slowly rose and at five years of age IgD was demonstrated in 61 per cent of the children which approaches the corresponding frequency in adults: 80 per cent.

When suspecting agammaglobulinemia during early infancy it is recommended that the levels of the different immunoglobulins are followed with repeated determinations. This requires a sensitive and accurate quantitative immunological method and paper electrophoresis and even immuno-electrophoresis must be considered unsatisfactory for this purpose. Such an example is given by Kouvalainen [8] in a study of children with frequent or fulminating infections. In this study immunoelectrophoresis was used to show that IgM could not be demonstrated in 12.5 per cent of the children below the age of 6 months. In the same group IgA could not be demonstrated in 68 per cent of the children and at the age of 1–2 years only 50 per cent of the children had demonstrable IgA. However the author pointing out the relatively low sensitivity of immunoelectrophoresis reaches the conclusion that this method is very suitable for clinical use. We feel that when immunological

deficiency conditions are suspected immunoelectrophoresis may be used as a screening method but when the immunoglobulin levels seem to differ from normal quantitation by a sufficiently sensitive and accurate method should be made.

## Summary

The concentrations of the four immunoglobulins (IgG IgA, IgM and IgD) have been studied in sera of 172 healthy children from the age of 6 weeks to 5 years. The method used was a single radial immunodiffusion technique according to Mancini with small modifications. The results obtained have been compared with those found in umbilical cord sera and with the average levels in healthy adults. The average concentration of IgG was lowest at the age of 4½ months i.e about one third of adult level. At the age of 1 year the average level of IgG was scarcely one half of adult level and at the age of 3–5 years about two thirds of the adult level. Low values in isolated healthy individuals were noted In cord sera IgA could only be demonstrated in one child out of 16 At the age of 1 year an average IgA level of about 13 per cent of adult level was found with remarkably high individual variation. IgM was found in all cord sera with an average concentration of barely 10 per cent of adult level At the age of 3 months the IgM values were about one half of adult level and at the age of 1 year approximately 70 per cent.

With one exception IgD could not be demonstrated until the age of 1 year Subsequently IgD was found in more and more children with advancing age At the age of 5 years IgD was found in 61 per cent of the children compared with 80 per cent of adults.

## Acknowledgements

The authors wish to express their thanks to Mrs. Stina Söderberg, Miss Birgitta Larsson and Miss Britt Ytterberg for skillful technical assistance We also thank Nurse Allie Håkansson and Mrs. Katarina Dellinder for their help at the Baby Welfare Center and Dr D S. Rowe University of Birmingham, England, who kindly provided the specific anti IgD sera.

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# Acute Toxoplasmosis in Childhood

by R. J HARRIS

*Toxoplasmosis Gondii* is a unicellular organism existing in 2 forms, proliferative and pseudocysts. The proliferative form is seen in acute toxoplasmosis, and is crescentic in shape The cystic form is seen during chronic infections more commonly in the eye brain, myocardium or muscle It is this form which is seen in congenital toxoplasmosis.

Until the advent of the Sabin Feldman dye test [22] reports of cases were sporadic, and mainly of the congenital variety [*0] although in 1942 Pinkerton & Henderson described a case of acute toxoplasmosis resembling Rocky Mountain Spotted Fever [20] In recent years a number of reports have appeared describing various syndromes of the acute disease [2 4 8 1* 23].

It is the purpose of this report to describe a case of acute toxoplasmosis in a _ year old boy and to discuss the main features of the acute infection with particular reference to one or two less well known aspects.

## Case Report

A boy aged years and 3 months, w admitted to hospital with a 5-day history of "moniliasis" of the mouth associated with anorexia and pyrexia. There had been no response to Dequadin and Nystatin. Previous to this episode, he had been perfectly well and his mother father and 4 siblings had also been well.

On examination he was noticed to have severe infective stomatitis, with cervical, axillary and inguinal lymphadenopathy and hepatomegaly the liver being smooth and firm. Nasopharyngeal swabs yielded no significant pathogenic bacteria, but a swab from his mouth yielded Staph. aureus, sensitive to Erythromycin. Haemoglobin concentration was 13.2 g/100 ml, sedimentation rat 34 mm/hr (Wintrobe) total whit cell count 8,200/mm with a slight eosinophilia (T ble 1). Heaf tuberculin tests, Wasserman reaction, Brucella agglutination Widal reaction and Paul Bunnell gglutination were all negative

Treatment with Glycerol and Thymol mouth washes and Erthromycin for 5 days improved his stomatitis, but two weeks later the lymphadenopathy was increasing, splenomegaly ppeared, and he developed mor billiform rash followed by the appearance of aphthous ulceration in the mouth At this time the toxoplasmosis dye test was reported as posit ve at 1 2048, and this was still the titre two weeks later

A suspension of material from a cervical lymph gland was inoculated intraperitoneally into known toxoplasma free mice and the parasit was isolated from the mice cerebral t sue one month later (Fig 1)

The hild remained well after the bove episode had subsided over the course of ten days. An ophthalmological opinion t this

TABLE 1 *Differential white cell count during course of illness*

| Date | 23.7 | 26.8 | 10.10 | 4.11 |
|---|---|---|---|---|
| Total WBC/mm³ | 8,200 | 9 600 | 10,100 | 12,000 |
| % Seg. neutrophils | 38 | 59 | 36 | 36 |
| % Juvenile forms | 2 | 6 | — | 2 |
| % Monocytes | 4 | 2 | 2 | 4 |
| Lymphocytes | 46 | 26 | 42 | 46 |
| Eosinophils | 10 | 12 | 20 | 12 |

TABLE 2. *Dye test titres in family of child with acute toxoplasmosis.*

| | | Initial titre | Titre after 6 weeks |
|---|---|---|---|
| Mother | | Negative | Not repeated |
| Father | | +(1 16) | Not repeated |
| Female sibling | (aet. 1) | Negative | Negative |
| Male sibling | (aet. 8) | +(1 128) | +(1 32) |
| Male sibling | (aet. 7) | +(1 128) | (1 128) |
| Male sibling | (aet 9) | +(1 128) | +(1 128) |

point showed chorioretinitis changes present which might be consistent with a diagnosis of healed toxoplasmosis (Mr C. A. Brown)

H was treated with Pyrimethamine and Sulphadiazine initially but the latter was discontinued as he developed an allergic type of rash. H completed a two-week course of Pyrimethamine and left hospital clinically satisfactory with some cervical lymph gland enlargement but no hepatosplenomegaly or stomatitis. The toxoplasmosis dye test was positive to a titre of 1 512 at this time H has remained clinically well for 12 months.

*Family investigations*

Table 2 shows the result of toxoplasmosis dye tests performed on other members of the family There was no clinical evidence of past or present infection with toxoplasmosis in any members of the family Mice infesting

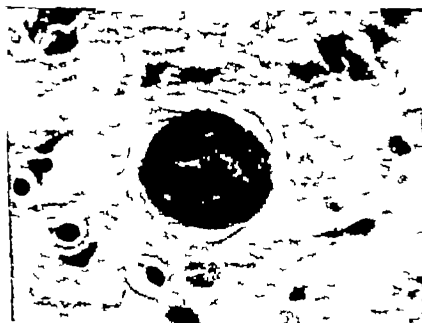


FIG 1 Photomicrograph of mouse cerebral tissue showing toxoplasma cyst following intraperitoneal inoculation of lymph gland extract from infected child Note particularly the lack of surrounding tissue reaction 1 ×0.

the house were caught alive, and fresh brain and right ventricular blood were examined from each of them. There was no evidence of infection with toxoplasmosis in any of the mice examined

## Discussion

The case of acute toxoplasmosis in a two year old child described has a number of unusual and interesting features. Lymphadenopathy is usually the most striking feature of acute toxoplasmosis. It may occur associated with a generalised febrile illness, as in this instance or indolent gland enlargement only may be noted [1]. Slim [23] noted that a large proportion of his cases presented with lymphadenopathy in particular associated with slight splenomegaly and pharyngitis, giving the disease a remarkable resemblance to glandular fever with which it may easily be confused. Lymph nodes are the best site from which the organism can be isolated

Stomatitis is an infrequently appreciated feature of acute toxoplasmosis. It was discussed in 1950 by Garin [12], who suggested that more attention be paid to "aphthous stomatitis" which is a clinical entity of somewhat mysterious aetiology Most cases have been shown to be associated with the virus of herpes simplex and in the child under discussion this may have been the situation the stomatitis having nothing at all to do with toxoplasmosis. However in obscure cases of stomatitis toxoplasmosis should always be considered. Pharyngitis is also a well recognised feature of acute toxoplasmosis, and may vary from a simple "sore throat to an ulcero-necrotic pharyngitis

Rashes in acute toxoplasmosis can take *many forms*, ranging from acute feverish exanthematous conditions resembling Rocky Mountain Spotted Fever [20] to purpuric and morbilliform rashes. Desmonts [5] described 10 cases of acute toxoplasmosis, 3 of whom had morbilliform rashes similar to the described case and 1 a purpuric rash. Once more the resemblance to glandular fever is apparent.

Although chorioretinitis is usually considered as one of the chief features of congenital toxoplasmosis (an average estimate being that 90% of cases demonstrate it), it also occurs quite commonly in acute toxoplasmosis [25] The condition may be almost symptomless, or be associated with clinical signs of generalised disease as in the child under discussion. Campbell & Clifton [2] describe a family with acute toxoplasmosis showing different stages in the development of chorioretinitis in the affected patients. Recurrent attacks of chorioretinitis result from cyst rupture in the retina

Haematological changes are manifold. The case under discussion showed eosinophilia and in six years 1957–1963, 6 proven cases of acute toxoplasmosis were seen in Bristol 4 of whom demonstrated marked eosinophilia The reason for this is not clear but rupture of cysts in the retina has been shown to give rise to necrotising lesions suggesting hypersensitivity [ 1], which may occur in the acute infection giving rise to the eosinophilia 7 out of Desmonts 10 cases also showed eosinophilia [5]

In addition to eosinophilia Desmonts also described the presence of abnormal mononuclear cells, resembling those found in glandular fever Slim [23] and Beverley & Beattie [1] all describe lymphocytosis but there appears to be no change in

number or character of neutrophil poly morphs in reported cases.

The family studies of the case under discussion are in keeping with the work of Huldt & Lagercrantz [16], who discovered that familial infection was very common among 120 families studied. This points to past infection, as there is now very little doubt that a positive dye test titre implies that the person has, t some time in the past, had toxoplasma infection, and, further more, that many of these cases are asymptomatic [9 10, 11].

It is not clear how infection reaches man. There is circumstantial evidence of a possible transmission between certain animals and man, with dogs, particularly those with distemper [3], cats [10, 17], rats and mice [11], pigeons [9], and sheep [13] among the more commonly implicated ones. The only known animal contacts of the family under discussion ere mice, and those infesting the house showed no evidence of infection. Arthropod ectors, however have by no means been excluded [14]. With regard to animals show ing positive dye test titres, Hartley [14] feels that both humans and animals may be infected from common source. His own work with ewes [13] leads him to believe that congenital infection may be the common method of natural transmission, the parasit migrating to certain sites of predilection in the body and there remaining dormant until activation by certain imper fectly understood stimuli. This spread plus the hypersensitivity theory of Sabin [21] may explain some cases of acut toxoplasmosis, and this idea is further supported in the case under discussion by the presence I healed chorioretinitis, eosinophilia, and evi dence of familial infection.

Evidence for other means of acquisition of the parasit is not convincing, lthough Cathie [4] has isolated toxoplasma organisms from the saliva of a child who presented lini cally with cervical lymphadenopathy sug gesting spread via a respiratory rout and Joseph et al. [19] ha recovered organisms from the mesenteric glands of children with mesenteric adenitis, suggesting ingestion as a possible means of spread. Interest in the ingestion theory has been revived recently by work of Hutchison [17] who showed that some form of toxoplasma is capable of passing into the environment in faeces of cats artificially infected with toxoplasma cysts. This form remained viable in tap water for 1 months, and caused infection when ingested by mice.

Evidence for successful treatment of toxoplasmosis is based mainly on animal experi ments. There is evidence that treatment with pyrimethamine and sulphadiazine in combi nation early in the acute disease may be curative [6, 18] Sulphones have also been shown to be active [24] but their toxicity limits their use to patients who cannot tolerate sulphonamides. Eyles & Coleman [7] showed that only 4,4-diaminodiphenyl sul phone is active enough to be of any practical value

The only definite diagnosis of toxoplasmosis can be made from isolation of the organism as described. A high dye test titre is significant, particularly if a rising titre can be demonstrated. Thus in cases presenting with any of the features de scribed, a very useful preliminary screening test is a toxoplasmosis dye test, which can be repeated, if necessary after weeks to demonstrat a rising titre

With regard to pregnancy if the disease is suspected on clinical grounds, speed in supply of adequate therapy is essential as it is known that the disease has a parasit aemia, and that the parasites cause a placentitis and omphalitis [15], with subsequent foetal infection.

## Summary

A case is described of acute toxoplasmosis, with a number of interesting features, in a 2 year-old boy The discussion

concerns the different manifestations of human infection with toxoplasmosis, and a discussion on the epidemiology and diagnosis of the disease

It is concluded that many problems remain in this enigmatic disease some of which are. Closer study of environmental conditions and the relationship with animal contacts Mode of communication of infection to man. The incidence of toxoplasmosis in unexplained febrile illnesses, especially those associated with lymphadenopathy or abdominal pain.


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# The Blood Volume of the Newborn Infant Delivered by Caesarean Section

by ALICE C. YAO A. WIST and JOHN LIND

While there are many reports that cautioned against early cord clamping, especially in regards to prevention of respiratory distress syndrome [5, 14, 17 19 21 30 31], there is also ample evidence that placental transfusion may be hazardous [2, 7 9 10, 24, 25].

James has recently stated that for the present, it is reasonable to regard the distribution of blood between the fetus and the placenta during intrauterine life as being physiological for both fetus and newborn rather than regarding blood in the placenta as the infant s birthright [18]

Our present study is designed as an extension of our studies on the rate of placental transfusion [39] in normal deliveries. Blood volume measurements were determined in babies born by caesarean section, where uterine contractions and breathing of the baby important factors in effecting placental transfusion, are non acting.

In this way we thought we might find out the pproximate blood volume of the fetus at term in utero

## Subjects and Method

### Subjects

16 newborn infants delivered by caesarean section t the Midwifery Institute, Helsinki, Finland. The gestational age ranged from 38 to 41 weeks, except in one case, A, No. 3 (see Table 2) 35 weeks, where the last menstrual period was not certain. This baby weighed 2600 g at birth. The birth weight ranges from 2600 g to 4040 g except two cases (A, No. 1) 2180 g and (B No. 1) 2420 g The latter two cases were f normal gestational age, but premature by weight. All babies appeared normal on physical examination.

Indications for caesarean section were usually a combination of two or more causes. But depending on the chief or immediat reason, it can be grouped as fetal or maternal (Table 2) Fetal distress or asphyxia was diagnosed in all five cases in group B by presence f fetal bradycardia with irregular rhythm and meconeum stained amniotic fluid. Maternal indications were varied as shown in Table 2. There were no significant hemorrhages in the cases with placenta praevia and ablatio placentae

All mothers received 0.5 mg atropine one-half hour before the operation, and thiopental 250 mg to 350 mg given intravenously followed by succinylcholine 75 mg before intubation. $N_2O$ ether and O inhalations were given a few minutes before birth, Methergine 0.2 mg was given after delivery of the babies.

All operations were performed by one operator except in three emergency cases. Uniform technique was applied whereby after the low cervical transverse incision the amniotic sac was opened and fluid released. The operator then placed his hand int the uterine cavity in search for a free loop of cord which was brought to the incision,

TABLE 1

| Case No. | Birth weight (g) | Body length (cm) | Age (min) | Apgar score at birth | Venous Hematocrit % | Blood volume corrected (ml) | Blood volume corrected ml/kg | Red cell volume ml | Red cell volume ml/kg | Plasma volume ml | Plasma volume ml/kg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A Nonasphyxiated* | | | | | | | | | | | |
| 1 | 180 | 46 | 50 | 7 | 59 | 139 | 64 | 71 | 33 | 68 | 31 |
| 2 | 2600 | 47 | 45 | 10 | 55 | 186 | 72 | 89 | 34 | 97 | 37 |
| 3 | 2600 | 48½ | 180 | 10 | 60 | 142 | 55 | 74 | 29 | 68 | 26 |
| 4 | 2750 | 48½ | 45 | 7 | 45 | 214 | 78 | 89 | 32 | 125 | 45 |
| 5 | 3330 | 51 | 55 | 10 | 50 | 202 | 60 | 104 | 31 | 98 | 29 |
| 6 | 3440 | 52 | 35 | 8 | 57 | 235 | 68 | 116 | 34 | 119 | 34 |
| 7 | 3620 | 5 | 60 | 9 | 49 | 245 | 68 | 104 | 29 | 141 | 39 |
| 8 | 3700 | 51 | 50 | 10 | 52 | 215 | 59 | 97 | 26 | 118 | 32 |
| 9 | 3800 | 52 | 60 | 7 | 52 | 235 | 62 | 106 | 28 | 129 | 34 |
| 10 | 4040 | 51 | 30 | 9 | 57 | 264 | 65 | 131 | 33 | 133 | 33 |
| 11 | 3630 | 51 | 55 | 8 | 51 | 273 | 75 | 121 | 33 | 152 | 42 |
| 12 | 3700 | 51 | 40 | 8 | 55 | 263 | 71 | 120 | 34 | 137 | 37 |
| 13 | 4930 | 52 | 45 | 9 | 52 | 329 | 67 | 149 | 30 | 180 | 37 |
| *B. Asphyxiated* | | | | | | | | | | | |
| 1 | 2430 | 49 | 100 | 4 | 60 | 226 | 93 | 118 | 49 | 108 | 44 |
| 2 | 2990 | 49 | 45 | 3–4 | 60 | 310 | 104 | 162 | 54 | 148 | 50 |
| 3 | 3040 | 51 | 40 | 3 | 56 | 249 | 81 | 121 | 39 | 128 | 42 |
| 4 | 3930 | 50½ | 55 | 4 | 57 | 320 | 82 | 159 | 41 | 161 | 42 |
| 5 | 3900 | 52½ | 35 | 3 | 50 | 354 | 91 | 154 | 39 | 200 | 51 |

clamped, cut and the baby immediately extracted. The times of birth of the whole baby and first breath were timed in reference to cord clamping with a stopwatch by one of us.

The babies airways were sucked out by a nasogastric catheter routinely and graded by Apgar score All five babies in group B with Apgar score of 4 or lower necessitated laryngeal intubation and short period of positive pressure breathing with $O_2$, varying from a few minutes to 20 minutes. N medi cations were used in any baby Apgar scores in group A babies were 7 or more

*Method*

Blood volume determinations were carried out using the Volemetron method, modified for use in newborn, using $^{125}$I tagged human serum albumin [20 28].

Radioactive $^{125}$I tagged albumin was used for the advantages of lower radiation effects and longer half-lif [22] Dosage used ranged from 0.15 microcurie to 0.25. This was given via a scalp vein and flushed with 2 ml normal saline through a 3-way stopcock; 5 minutes later a 2 ml (postmix) blood sample was withdrawn from the femoral vein. A control (premix) blood sample was btained from the umbilical vein after the cord was clamped.

The radioactivity of the dose syringe was measured before and after injection and premix and postmix blood samples were also counted and blood volume mechanically calculated. Correction factors for difference of total body and venous hematocrit were utilized t derive the corrected blood volume and Rbc volume as follows:

$$*CBV = BV \frac{1 - \text{Ven. hematocrit \%}}{1 - 0.87\ \text{Ven. hematocrit \%}} \ (\text{ml})$$

$$\text{Rbc V} = \text{CBV} \cdot 0.87\ \text{Ven. hematocrit \%}\ (\text{ml})$$

Prepared by Aktiebolaget Atomenergi, Studsvik, Sweden, in 10 ml vials with concentration of 1 microcurie per ml.

CBV = corrected blood volume; BV blood volume as determined directly by volemetron; Rbc V = red cell volume.

TABLE –

| Case No. | Mother: Age (years) | Mother: Parity | Gestation age (weeks) | Prenatal history | Labor before C-section | Indication for C-section |
|---|---|---|---|---|---|---|
| *A. Nonasphyxiated* | | | | | | |
| 1 | 24 | 1 | 41 | Preeclampsia, BP 166/120 | 27 h | Uterine inertia preeclampsia |
| 2 | 30 | 2 | 38 | Uneventful | 2½ h | Placenta Praevia complete |
| 3 | 20 | | [illegible] | Anemia at 8th month, treated with iron pills, slight bleeding 3 days prior to delivery | 12 h | Abruptio placenta[a] rigiditas oris uteri |
| 4 | 23 | 1 | 40 | Uneventful | 42 h 18 m | Prolonged labor[a] Maternal exhaustion |
| 5 | 28 | 2 | 40 | Uneventful | 0 | Contracted pelvis Repeat C/S |
| 6 | 19 | 1 | 41 | Uneventful | 60½ h | Contracted pelvis |
| 7 | 19 | 1 | 41 | Uneventful | 29½ h | Uterine inertia |
| 8 | 39 | 1 | 40 | Uneventful | 18 h 20 m | Maternal exhaustion elderly primigravida |
| 9 | 32 | 3 | 38 | Pre-eclampsia BP 150/100 | 29 h 4 m | Pre-eclampsia[a] ? fetal distress |
| 10 | 23 | 1 | 40 | Uneventful | 45½ h | Rigitas oris uteri[a] Maternal exhaustion |
| 11 | 17 | 1 | 40 | Uneventful | 9 h | Uterine inertia |
| 12 | 24 | 1 | 40 | Pre-eclampsia, anemia at 8th mo. | 6 h | Pre-eclampsia[a] |
| 13 | 23 | 1 | 40 | Threatened abortion, 3rd month | 14 h | Uterine inertia[a] |
| *B. Asphyxiated* | | | | | | |
| 1 | 31 | 1 | 41 | Rh negative mother but no significant titer. Otherwise uneventful | 34 h 40 m | Fetal distress[a] elderly primipara, uterine inertia |
| 2 | 32 | 2 | 40 | Severe pre-eclampsia BP 270/170, poorly controlled edema | ½ h | Fetal distress[a] toxemia |
| 3 | 29 | 1 | 39 | Uneventful | *8½ h | Fetal distress[a] Maternal exhaustion |
| 4 | 23 | | 41 | Pre-eclampsia BP 180/110 | 18 h | Fetal distress[a] pre-eclampsia |
| 5 | 32 | 1 | 40 | Threatened abortion, 3rd mo | 20 h | Fetal distress[a] Mental presentation |

[a] Chief indication.

The venous hematocrit was measured by the microcapillary technique in triplicate [16].

Each infant received one drop of Lugol solution daily for three days.

The measurements were performed within the first hour of life in all except case B, No. 1 at 1½ hours, and A, No. 3 at age 3 hours.

## Results

The blood volume in these babies, though delivered with cord clamped in the same manner before birth clearly falls into two distinct categories depending on the chief indication of caesarean section.

In the thirteen babies delivered by

TABLE 1

| Case N | Birth weight (g) | Body length (cm) | Age (min) | Apgar score at birth | Venous Hematocrit % | Blood volume corrected (ml) | Blood volume corrected ml/kg | Red cell volume ml | Red cell volume ml/kg | Plasma volume ml | Plasma volume ml/kg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. Nonasphyxiated* | | | | | | | | | | | |
| 1 | 2180 | 46 | 50 | 7 | 59 | 139 | 64 | 71 | 33 | 68 | 31 |
| 2 | 2600 | 47 | 45 | 10 | 55 | 188 | 72 | 89 | 34 | 97 | 37 |
| 3 | 2600 | 46½ | 180 | 10 | 60 | 142 | 55 | 74 | 29 | 68 | 26 |
| 4 | 2750 | 48½ | 48 | 7 | 48 | 214 | 78 | 89 | 33 | 125 | 45 |
| 5 | 3380 | 51 | 55 | 10 | 59 | 203 | 60 | 104 | 31 | 96 | 29 |
| 6 | 3460 | 52 | 35 | 9 | 57 | 235 | 68 | 116 | 34 | 119 | 34 |
| 7 | 3620 | 52 | 60 | 8 | 49 | 45 | 68 | 104 | 29 | 141 | 39 |
| 8 | 3700 | 51 | 50 | 10 | 52 | 215 | 58 | 97 | 26 | 118 | 32 |
| 9 | 3600 | 52 | 60 | 7 | 5 | 225 | 62 | 106 | 28 | 129 | 34 |
| 10 | 4040 | 51 | 30 | 9 | 57 | 264 | 65 | 131 | 32 | 133 | 33 |
| 11 | 3630 | 51 | 55 | 8 | 51 | 273 | 75 | 121 | 33 | 152 | 42 |
| 12 | 3700 | 51 | 40 | 8 | 55 | 263 | 71 | 126 | 34 | 137 | 37 |
| 13 | 4930 | 52 | 45 | 9 | 52 | 329 | 67 | 149 | 30 | 180 | 37 |
| *B. Asphyxiated* | | | | | | | | | | | |
| 1 | 2430 | 49 | 100 | 4 | 60 | 226 | 93 | 118 | 49 | 108 | 44 |
| 2 | 2990 | 49 | 45 | 3–4 | 60 | 310 | 104 | 162 | 54 | 148 | 50 |
| 3 | 3040 | 51 | 40 | 3 | 56 | 249 | 81 | 121 | 39 | 128 | 42 |
| 4 | 3930 | 50½ | 55 | 4 | 57 | 320 | 82 | 159 | 41 | 161 | 42 |
| 5 | 3900 | 52½ | 35 | 3 | 50 | 354 | 91 | 154 | 39 | 200 | 51 |

clamped, cut and the baby immediately extracted. The times of birth of the whole baby and first breath were timed in reference to cord clamping with a stopwatch by one of us.

The babies airways were sucked out by a nasogastric catheter routinely and graded by Apgar score. All five babies in group B with Apgar score of 4 or lower necessitated laryngeal intubation and short period of positive pressure breathing with $O_2$, varying from a few minutes to 20 minutes. No medications were used in any baby. Apgar scores in group A babies were 7 or more.

### Method

Blood volume determinations were carried out using the Volemetron method, modified for use in newborn, using $^{131}$I tagged human serum albumin [20, 38]

Radioactive $^{131}$I tagged albumin was used for the advantages of lower radiation effects and longer half-life [22]. Dosage used ranged from 0.15 microcurie to 0.25. This was given via a scalp vein and flushed with 2 ml normal saline through a 3-way stopcock; 5 minutes later a 2 ml (postmix) blood sample was withdrawn from the femoral vein. A control (premix) blood sample was obtained from the umbilical vein after the cord was clamped.

The radioactivity of the dose syringe was measured before and after injection and premix and postmix blood samples were also counted and blood volume mechanically calculated. Correction factors for difference of total body and venous hematocrit were utilized to derive the corrected blood volume and Rbc volume as follows:

$$*CBV = BV \frac{1 - \text{Ven. hematocrit \%}}{1 - 0.87 \text{ Ven. hematocrit \%}} \text{ (ml)}$$

$$\text{Rbc V} = \text{CBV} \quad 0.87 \text{ Ven hematocrit \% (ml)}$$

Prepared by Aktiebolaget Atomenergi, Studsvik, Sweden, in 10 ml vials with concentration of 1 microcurie per ml.

CBV = corrected blood volume; BV = blood volume as determined directly by Volemetron; Rbc V = red cell volume.

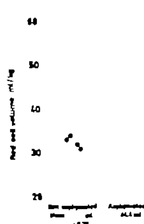



Fig. 1. Red cell volume of 18 newborn infants delivered by caesarean section in the first hours of life.

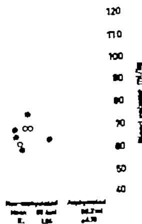



Fig. 2. Blood volume of 18 newborn infants delivered by caesarean section in the first hours of life.

babies, we obtained a uniformly small blood volume in the absence of fetal distress (group A). This blood volume probably approximates that which the fetus at term has in utero. The condition at birth here was such that factors which bring about or influence placental blood transfer to the baby following normal vaginal delivery were not acting. Those factors include the change in the feto-placental vascular pressure relationship favoring blood flow to the baby uterine contraction, breathing of the baby size and patency of the umbilical vessels and effect of gravity

Furthermore, the finding of a similarly small and comparable blood and red cell volumes in the six normal vaginally delivered infants whose cords were clamped before complete birth and first breath was of extreme interest (Table 3). These six infants had a tight coil of cord around their neck arresting the umbilical circulation. They too will have a blood volume approximating that in utero or that before any placental transfusion.

TABLE 3 *Blood volumes red cell volumes and plasma volumes of six newborn infants whose cords were clamped before complete vaginal delivery because of tight coils around the neck*

| Case No. | Birth weight (g) | Body length (CM) | Age (min) | Blood volume (ml) | Blood volume (ml/kg) | Red cell volume (ml) | Red cell volume (ml/kg) | Plasma volume (ml) | Plasma volume (ml/kg) | Venous hematocrit % |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2900 | 50 | 55 | 180 | 64 | 71 | 25 | 109 | 39 | 45 |
| 2 | 3200 | 50 | 50 | 265 | 85 | 97 | 30 | 168 | 52 | 42 |
| 3 | 3200 | 51 | 65 | 252 | 79 | 118 | 37 | 134 | 42 | 54 |
| 4 | 3400 | 51 | 180 | 208 | 61 | 105 | 31 | 103 | 30 | 53 |
| 5 | 4070 | 52 | 55 | 279 | 69 | 136 | 34 | 143 | 36 | 56 |
| 6 | 4060 | 50 | 45 | 238 | 59 | 97 | 24 | 141 | 35 | 47 |

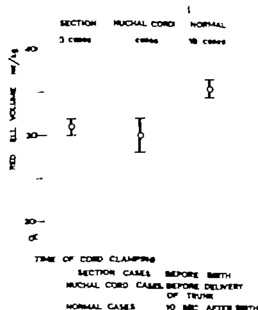



Fig. 3. Comparison of the mean red cell volume in 13 newborn infants delivered by caesarean section; 6 infants delivered vaginally with the cord round the neck, and 15 normal infants delivered spontaneously; at different times of cord clamping

Using the red cell volume as a guide to the amount of placental transfusion [38], comparison of the caesarean section group A cases, the nuchal cord cases, and normal cases with cords clamped ten seconds after birth, revealed *significant transfusion* soon after normal birth (Fig 3) In other words our findings support the assumption that for placental transfusion to occur pressure gradients between the fetal and placental vascular systems have to exist and uterine contraction as a propelling force patency and size of the umbilical vessels, expansion of the baby's lungs are all important factors under normal condition.

However with the findings of high blood volumes in the five asphyxiated babies, one cannot help but ask this question. Is it possible that these babies, with antepartum *distress have a bigger blood volume* in utero? Or do they acquire it after birth? Since the umbilical cords were clamped the same way as those of group A, before birth, it is not possible for placental transfusion to occur in the usual sense It is conceivable that in the presence of fetal asphyxia, compensatory mechanisms are called into play resulting in in*creased fetal cardiac output and umbilical* flow in an effort to achieve more gaseous exchange or O uptake through the placental circulation [4, 8 11 12, 18 23, 29]. It is possible that as a result of these *compensatory responses, there is a re*distribution of the total blood volume between the fetus and placenta. Perhaps, as a consequence of a relatively faster rate of umbilical flow there is an increased amount of blood flow through the fetus. The vascular bed capacity in the fetal side may have expanded to accomodate a relatively bigger blood volume at this state

When asphyxia is of severe degree, release of catecholamines from the fetal adrenals may occur causing umbilical vasoconstriction, more so in the arteries than in the vein. Gross studies on umbilical vessels *of asphyxiated babies showed* increased number of vasoconstriction in the arteries but not so much in vein [40] This, together with increased umbilical flow may result in a relatively greater volume in the fetal side.

It is hard to know what exactly happened in our five cases. Another possible mechanism involved may be the remobilization of the normally sequestered blood in reserve in the baby notably in the liver and spleen, into circulation. Thus, resulting in a greater circulating blood volume This is likely to happen with the stress of asphyxia continuing after birth [30]

With asphyxia, the likely possibility of increased permeability of peripheral blood vessel wall leading to rapid plasma exudation, and consequently rapid leakage of the albumen tagged tracer [131]I into the extravascular space resulting in faulty high blood volume has to be considered. The fact that both red cell and plasma volumes were high contradicted rapid plasma exudation. The short time between injection of tracer and blood sampling makes this possibility of error less likely. Besides, multiple blood sampling in one of the asphyxiated cases showed tracer disappearance rate to be comparable to normal cases. This also does not favor poor mixing due to peripheral vasoconstriction as the source of faulty blood volume measurement.

It is interesting to note that none of the five babies showed any signs of heart failure or shock within the immediate hours after birth in spite of their initial difficulties. They all appeared, clinically as cases of asphyxia livida at birth. It should be most interesting to find out whether the blood volume of babies with asphyxia pallida is different. From the findings of the above five cases, it would seem that the usual practice of delayed cord clamping in a livid, asphyxiated newborn should be cautioned against since overloading an already overloaded circulation can be hazardous.

All of the thirteen cases in group A also had an uneventful course.

## Summary

The blood volumes of eighteen babies delivered by caesarean section with cord clamping in utero were studied.

The results in thirteen, operated on maternal indication, showed a low blood and red cell volume comparable to six cases born vaginally where nuchal cords were clamped before birth of the body. These blood volumes were considered to approximate the fetal blood volume in utero at term.

Five cases with diagnosed asphyxia antepartum, were found to have high blood and red cell and plasma volumes. The possible explanations were discussed.

## Acknowledgement

We are very grateful to Doc. W. Nyberg, Head of Laboratory Midwifery Institute, Helsinki, Finland, for his generosity and kindness in giving us the space, facilities, and all possible assistance from his staff in accomplishing this work. We also wish to thank all the doctors, midwives, operating room and nursing staff and baby nurse Miss A. Nummelin for their kind co-operation and help during this work.

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# The Adrenal Cortical Glucocorticoid Function in Asthmatic Children

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Adrenocortical hyperfunction or hypofunction may be suspected to exist in asthmatic children. Adrenocortical stimulation may be caused by the severe respiratory distress of asthmatic paroxysms or by the chronic stress imposed by the recurrence of such attacks and the debilitating effect of chronic illness. On the other hand the severe asthmatic attacks may cause pituitary-adrenal exhaustion or the debilitating effect of the chronic illness may have interfered with the nutrition and function of the pituitary-adrenal axis. Also it is possible that a state of pituitary adrenal insufficiency may have rendered the asthmatic children more susceptible to the effects of sensitizing antigens similar to what occurs in hypophysectomized or adrenalectomized animals [9 14].

With these points in mind the present work was carried out aiming to determine the glucocorticoid function of the pituitary-adrenal axis in asthmatic children. It is hoped that the results of this work may clarify the role played by the adrenal cortex in the pathogenesis of asthma and that they may help in explaining the causes of contradiction between the data offered by various workers interested in this subject [1–3 6 7 10 12, 16 18 21 23–30].

## Material

Two groups of children were studied:

A group of 16 normal healthy children aged 4 years or more [illegible] males and 8 females.

A group of 32 asthmatic children aged 4 years or more, 18 males and 14 females, in whom the onset of asthma dated back for at least two years. No child was included who had received steroid treatment in the last six months prior to admission.

## Method

Asthmatic children were classified into three categories: Severe, Moderate and Asymptomatic according to the severity of respiratory distress as indicated by clinical examination and ventilatory capacity tests.

The study of adrenocortical glucocorticoid function included:

1. Estimation of plasma 17-hydroxycorticosteroids (17-OHCS) by the method of Peterson *et al.* [19] utilizing the Porter Silber color reaction [20]. Cortrophin [4] in doses of 15 units in 500 ml 5 % glucose in water were in

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TABLE * *Plasma 1 -OHCS and their response to I V ACTH in asthmatic children.*

mcg/100 ml

| No. | Sex | Severe | | Moderate | | Asymptomatic | |
|---|---|---|---|---|---|---|---|
| | | Before ACTH | After ACTH | Before ACTH | After ACTH | Before ACTH | After ACTH |
| 1 | F | 1 | — | 16 | 26 | 7 | 26 |
| 2 | M. | 28 | — | 19 | 30 | 6 | 20 |
| 3 | F | 27 | — | 5 | — | — | — |
| 4 | M. | 20 | — | — | — | 18 | — |
| 5 | F | 28 | — | — | — | — | — |
| 6 | F | 20 | — | — | — | 6 | 16 |
| 7 | M. | 4 | — | 4 | 28 | 3 | 2 |
| 8 | M. | 26 | — | 19 | — | 8 | — |
| 9 | F | 28 | — | 4 | 12 | — | — |
| 10 | M. | 16 | — | 11 | — | 10 | — |
| 11 | M. | 13 | 35 | — | — | 8 | 46 |
| 12 | F | 22 | 43 | — | — | 6 | 20 |
| 13 | F | 18 | 40 | — | — | 6 | 16 |
| 14 | F | 18 | 34 | — | — | 9 | 28 |
| 15 | M. | 21 | 33 | — | — | | 9 |
| 16 | F | 17 | 28 | — | — | 4 | 18 |
| 17 | F | 20 | 2 | 3 | 10 | — | — |
| 18 | M. | — | — | 16 | 30 | 6 | 2 |
| 19 | F | — | — | 12 | 30 | | 29 |
| 20 | M. | — | — | 11 | 1 | 6 | 14 |
| 21 | M. | — | — | 18 | — | 6 | — |
| 22 | M. | — | — | 13 | 27 | 7 | 22 |
| Range | | 16–29 | 28–42 | 3–19 | 10–30 | 3–18 | 9–2 |
| Mean | | 22.8 | 34 9 | 11.3 | 20.6 | 6 7 | 1 2 |
| s.d. | | 4 2 | 4.8 | 4.8 | 10 1 | 3 4 | 6.5 |

in controls (15.2 mg. s.d. 5.), asymptomatic asthmatics (14.3 mg. s.d. 6.) and moderate asthmatics (16. mg. s.d. 8.4)

*Blood eosinophil counts*

The mean eosinophil counts of asthmatic children were significantly higher than control counts even when free from evidence of respiratory distress (asymptomatic). The percentile drop in blood eosinophils after exogenous ACTH administration was insignificantly different in control and asthmatic children (T ble 4 Graphs 4 and 5)

*Metopirone test*

Peak increase in total 24-hour urinary 17-OHCS excretion occurred on the day after metopirone administration in controls and asthmatics. However the peak values in controls (mean 13.5 mg s.d. 1) were significantly higher than in Asymptomatic asthmatics (6 4 mg d 3 0) Also the peak increase over pre metopirone values was significantly higher in controls (9 1 mg. s.d. 5) than in asymptomatic asthmatics (4 0 mg d 1.8)

*Effect of duration of illness*

In asthmatic children no significant correlation was found between the duration of their illness (in years) and their pre ACTH or post ACTH 17 OHCS levels in their response to metopirone

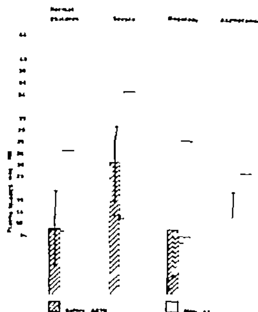


Fig. 1. Plasma 17-OHCS levels and their response to I.V. ACTH infusion.

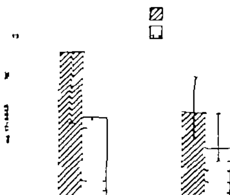


Fig. 2. Morning and evening plasma 17-OCHS levels in normal and asymptomatic asthmatic children.

**Table 3.** *Urinary 17-OHCS and their response to I.M. ACTH Z in asthmatic children.*

mg/m²/24 hours

| No. | Sex | Moderate | | Asymptomatic | |
|---|---|---|---|---|---|
| | | Before ACTH | After ACTH | Before ACTH | After ACTH |
| 3 | F | 3 6 | 12.1 | — | — |
| 6 | F | 5.1 | 27 4 | — | — |
| 7 | M. | — | — | 1.5 | 10.2 |
| 8 | M. | 7 0 | — | 1.9 | — |
| 9 | F | 1.5 | 8 0 | — | — |
| 15 | M. | — | — | 2.1 | 15.0 |
| 16 | F | 2.6 | — | 1 9 | 27 1 |
| 17 | F | 12.5 | 14.1 | — | — |
| 20 | M. | 4.6 | 10.6 | — | — |
| 1 | M. | 4.2 | 22.2 | 3.5 | 31 4 |
| 22 | F | 4.9 | 10.2 | 1 7 | 8.2 |
| 23 | F | 6.0 | 23 1 | 2.0 | 16.5 |
| 24 | F | 5.2 | 14.0 | 2.3 | 8.2 |
| 25 | M. | 3.9 | 13 0 | 2.5 | 6.9 |
| 26 | F | 4.6 | 9 7 | 2.2 | 5.6 |
| 27 | M. | 5.0 | — | 1.9 | 10.5 |
| 28 | M. | 2.6 | — | 1.3 | 7.5 |
| 29 | M. | 8.7 | 13.4 | 6.5 | 10.5 |
| 30 | F | 2.0 | 16.3 | 1.9 | 18 3 |
| 31 | M. | 4.0 | 27 7 | 2.0 | 21.5 |
| 33 | M. | 1 4 | 11.8 | 1 0 | 21.2 |
| Range | | 1.4–12.5 | 5.0–27 7 | 1 0–6.5 | 5–[illegible] |
| Mean | | 4.7 | 16 | 2.3 | 14 3 |
| S.D | | 2 6 | 8.4 | 1.3 | 6.2 |

## Discussion

In asthmatic children free from evidence of respiratory distress (asymptomatic) the mean 9.0 a.m. plasma 17 OHCS level was about 58 % and the mean 24-hour urinary 17-OHCS excretion was about 52 % the normal control values. This would reflect a state of partial adrenocortical glucocorticoid insufficiency the adrenals of the resting asthmatic children could produce a little more than half that amount of cortisol and cortisone that can be produced by the adrenals of healthy control children.

*Adrenocortical cortisol production may*

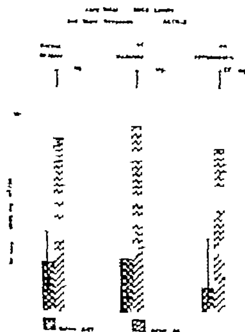


Fig. 3. Urinary total 17-OCHS levels and their response to i.m. ACTH Z.

**Table 4.** *Circulating blood eosinophils and their % drop after ACTH in asthmatic and healthy children*

| | Asthmatic Children | | | |
|---|---|---|---|---|
| | Severe | Moderate | Asymptomatic | Normal healthy children |
| *Eosinophils/cmm before ACTH* | | | | |
| Range | 231–935 | 110–973 | 104–704 | 33–517 |
| Mean | 640.2 | 533.1 | 440.5 | 215.8 |
| S.D. | 198.5 | 226.0 | 1 6.1 | 134.8 |
| *% drop after i. ACTH* | | | | |
| Range | 61–92 | 60–90 | 50–85 | 44–90 |
| Mean | 74.0 | [illegible] | 79.0 | 72.2 |
| S.D. | 10.7 | [illegible] | 10.6 | [illegible] |
| *drop after i.m. ACTH-Z* | | | | |
| Range | — | 85–99 | 87–99 | 82–100 |
| Mean | — | 94.2 | 94.4 | 96.5 |
| S.D. | — | 4.3 | 4.0 | 5.1 |

be reduced due to primary adrenal disease or otherwise may be secondary to lack of trophic hormone (pituitary ACTH) stimulation. When exogenous ACTH was



Fig. 4. Blood eosinophil counts and their response to ACTH.

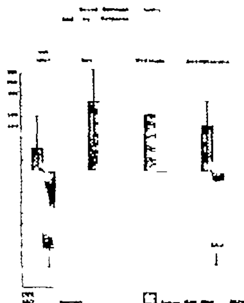


Fig. 5. Blood eosinophil counts and their response to i.m. ACTH Z.

Fig 1 Plasma 17-OHCS levels and their response to i.v ACTH infusion.

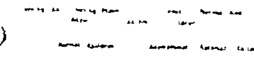


TABLE 3 *Urinary 17-OHCS and their response to I.M ACTH Z in asthmatic children*

mg/m²/24 hours

| N | Sex | Moderate Before ACTH | Moderate After ACTH | Asymptomatic Before ACTH | Asymptomatic After ACTH |
|---|---|---|---|---|---|
| 3 | F | 3.6 | 13.1 | — | — |
| 6 | F | 5.1 | 27 4 | — | — |
| 7 | M. | — | — | 1.5 | 10.2 |
| 8 | M. | 7.0 | — | 1.9 | — |
| 9 | F | 1.5 | 6.0 | — | — |
| 15 | M. | — | — | 2.1 | 18.0 |
| 16 | F | 2.6 | — | 1 9 | 27.1 |
| 17 | F | 12.5 | 14 1 | — | — |
| 20 | M. | 4.6 | 10 6 | — | — |
| 21 | M. | 4.2 | 23.2 | 3.5 | 21 4 |
| 22 | F | 4.9 | 10.2 | 1.7 | 8.2 |
| 23 | F | 6.0 | 23.1 | 2.0 | 16.5 |
| 24 | F | 5.2 | 14.0 | 2.3 | 8 2 |
| 25 | M. | 3.9 | 13.0 | 2.5 | 6 9 |
| 26 | F | 4.6 | 9 7 | 2.2 | 5 6 |
| 27 | M. | 5 0 | — | 1.9 | 19 5 |
| 28 | M. | 2.6 | — | 1.3 | 7.8 |
| 29 | M | 6.7 | 13 4 | 6.5 | 10.5 |
| 30 | F | 2.0 | 16.2 | 1.9 | 18.3 |
| 31 | M. | 4.0 | 27 7 | 2.0 | 21.5 |
| 32 | M. | 1 4 | 11.8 | 1 0 | 21.3 |
| Range | | 1 4–12.5 | 6.0–27 7 | 1.0–6.5 | 8.2–27.1 |
| Mean | | 4.7 | 16.2 | 2.3 | 14.3 |
| S.D | | 2.6 | 8 4 | 1.3 | 6.2 |

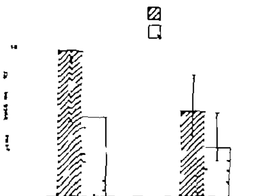


Fig 2. Morning and evening plasma 17-OCHS levels in normal and asymptomatic asthmatic children.

## Discussion

In asthmatic children free from evidence of respiratory distress (asymptomatic) the mean 9 0 a.m. plasma 17 OHCS level was about 58 % and the mean 24-hour urinary 17 OHCS excretion was about 53 % the normal control values. This would reflect a state of partial adrenocortical glucocorticoid insufficiency the adrenals of the resting asthmatic children could produce a little more than half that amount of cortisol and cortisone that can be produced by the adrenals of healthy control children.

Adrenocortical cortisol production may

ficiency in asthmatic children was not a sequence of their long-standing illness. On the other hand it is more probable that asthmatic children suffer from partial pituitary-adrenal insufficiency which may have rendered them more susceptible to anaphylactic challenge

The data presented in this work are similar to those reported by several workers [3, 8, 17 21 22 26–28] but different from those offered by many others [1 6 7 10, 12, 16 18, 22, 24 30]. The subnormal response of asthmatic children to metopirone is comparable to that obtained by Andersson [2]. If reliable specific methods of steroid determination and proper experimental design were followed, slight contradiction, if any would have existed between various workers.

### *Summary and Conclusions*

The adrenocortical glucocorticoid function was studied in 32 asthmatic children and 15 healthy control children of comparable sex and age

The mean plasma and urinary 17-OHCS levels in *asymptomatic asthmatic children* were a little more than half the mean levels in control children. However the rise in 17 OHCS levels and percentile drop in blood eosinophils produced by exogenous ACTH administration were *similar in both* groups. In asthmatic children suffering from moderate respiratory distress the mean plasma and urinary 17 OHCS levels were insignificantly different from those in control children. When ACTH was administered to asthmatic children they exhibited normal rise in their 17 OHCS values and drop in their blood eosinophil counts with marked amelioration of respiratory distress.

During severe asthmatic paroxysms the mean plasma *17 OHCS level* was almost double that of healthy children. Intravenous ACTH infusion to those children produced significant rise in their plasma 17-OHCS levels with significant improvement of their suffering.

*It was suggested* that asthmatic children suffer from a partially defective pituitary adrenal system which might have rendered them more susceptible to anaphylactic challenge. The subnormal rise in urinary 17 OHCS excretion which followed metapirone *administration to a few asymptomatic* asthmatic children may provide a further support of that assumption.

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# The Vasculature of the Developing and Mature Human Adrenal Gland

by BIÖRN IVEMARK, TORE EKSTRÖM and CURT LAGERGREN

As was shown by Kölliker in 1854 [6] the adrenal gland in man is supplied by arteries that divide outside the capsule into a number of branches which pass through the capsule and ramify centripet ally in the cortex and medulla. The com plexity of the vascular supply of the adrenal glands [10] is manifested in the difficulty of diagnosing pathologic condi tions in the glands by angiography From an article by Lever [8] it is evident that the vascular pattern of the fully developed gland is similar in most mammals. No study seems to have been made however of the development of the vascular architecture during the pre and postnatal growth of the adrenals.

In the course of a study of the neoplastic changes in the adrenal glands the need for comparison with the normal microangiographic vascular pattern was recognized [3]. The investigation was accordingly extended to cover the development of the adrenal glands.

## M terial

*Developing adrenal gland* The adrenal gland from 20 fetuses and newborn, all of them autopsy subjects, aged up to 6 weeks, were examined post mortem after injection of contrast medium, they comprised 16 fetuses aged 14–20 weeks and surviving 0–4 days and 4 full term infants living for up to 6 weeks. None of the deaths were from diseases involving changes in the adrenal glands.

*Mature adrenal gland* This series consisted mainly of surgical specimens from women that had undergone adrenalectomy for advanced mammary carcinoma (12 patients) Although some of the patients had received treatment that might have affected the structure of the adrenal glands it was con sidered that these glands were acceptable as a "normal" series. For the whole group the vascular architecture was found to be uni form. In 6 of the cases of mammary car cinoma it was possible to fill both glands with medium. At the examination of the prepared specimens 4 of the patients were found to have suprarenal metastases, 2 f them bilaterally The normal series was thus reduced t 12 adrenal glands from 8 women; they ranged in age from 39 to 64 years. In addition, one utopsy specimen was btained from 7 year-old child, bringing the total normal series up t 13 glands in which it may be assumed that the postnatal processes of involution and maturation had been arrested.

## Method

*Developing adrenal.* In contrast to the procedure for most of the adult specimens those of the fetuses and children were in jected with medium via the aorta after the

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kidneys and adrenal glands had been removed *en bloc* with the associated segment of the aorta from 2 cm cranially of the diaphragm to 2 cm distally of the renal arteries. A 7.5 per cent aqueous suspension of barium sulphate (Micropaque[1]) was injected at a pressure of about 80 mm Hg in a period of 30–45 minutes. There was usually considerable leakage from the vessels severed on removing the specimen, but acceptable filling was *obtained after appropriate ligation*. The best results were obtained in 2 cases in which the injection was made into the aorta after removal of the abdominal organs *en bloc*.

After radiography and fixing in formaldehyde the adrenals were dissected and cut into 2–3 mm slices, which were again radiographed suitable pieces with good visualization of the arteries were then embedded for microangiography and histologic examination [1 7 9].

*Adult normal subjects* Immediately after operation the specimens were freed of superficial fat, weighed, and injected with contrast medium. Because of the wide variation in the vascular supply of the adrenal glands and the ramification of the supplying arteries into numerous small branches prior to their entry into the organ, it was technically difficult to inject the medium via the arteries. As the glands have only 1 or 2 veins, all the glands from the cases of mammary carcinoma were filled with contrast medium through a suprarenal vein. Because of the characteristic nature of the vascular system of these glands, with fairly wide sinusoids between the arterial and venous sides, it was evident from pilot experiment that filling also of the arterial side adequate for the purpose of the study could be obtained. After fixation in formaldehyde the specimens were prepared in the same way as those of the fetuses and the newborn infants.

## Results

### *Developing adrenal*

The vascular pattern of the fetal adrenal gland was found to undergo a gradual change from the earliest stage examined (14th fetal week) until the postnatal involution and maturation were complete In the early fetal stages the injection of medium provided satisfactory filling of central, medullary vessels from which sinusoids radiated towards the capsule (Fig 1) During this stage of development the greater part of the gland consisted of the provisional fetal cortex, which was vascularized mainly from the center of the organ, out towards the periphery Filling of the capsular arteries was minimal However they increased in clarity with age and the medullary arteries became sparser (Figs. 2–4) When arteries occurred in the capsule small spray like formations were found in the peripheral parts of the cortex, corresponding histologically to the incipient differentiation of the zona glomerulosa. At the time of birth there was a narrow subcapsular border of such sprays, corresponding to a narrow zona glomerulosa (Fig 5) This widened further after birth, and with the formation of the zona fasciculata during the second month of life [11] sinusoidal sprays of adult type were visualized. In the present series, however this was not seen before 7 years of age (Fig 6) but since the period between 6 weeks and 7 years was not

Fig. 1 (A) Micro-angiogram of fetal adrenal gland (14 weeks) The vessels of the medulla and fetal cortex are clearly visualized, but not those of the capsule. 16. (B) Histologic section of the specimen in Fig 1A. Most of the gland is composed of provisional fetal cortex (The disruption of the cell cords is due to the hypotonicity of the contrast medium.) *Hematoxylin and eosin.* 16. *Fig* 2. (A) Micro-angiogram of fetal adrenal gland (fetal weight 910 g, age 27 weeks). Capsular arteries are just appearing and sinusoids are clearly depicted. 16. (B) Histologic section of the specimen in *Fig.* 2A. The zona glomerulosa has increased in thickness. *Hematoxylin and eosin.* 16.


1


1b

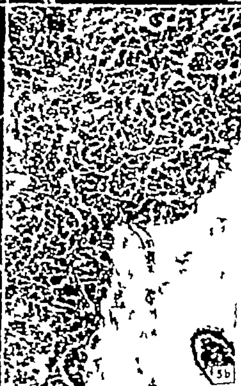

2a


2b


3


4


5a


5b

represented, it was not possible to ascertain the time at which the adult pattern was established.

*Adult normal specimens*

The histologic examination showed that the veins, sinusoids and medullary arteries were invariably well filled but the capsular arteries in only _ specimens.

The principal feature of the normal vascular pattern of the adrenal glands was the spray like formation of sinusoids. These were extremely dense, parallel and straight, and entered the vein at right angles. The veins joined up as they approached the larger central medullary vessels. The arteries leading towards the sinusoids ran in the same direction as them, and were given off at right angles from the slightly wider capsular nd medullary arteries. At the poles of the adrenal glands the sinusoids converged towards central medullary veins. No wide arteries were found in the gland, whose supply usually appeared *to come from multiple small* capsular arteries.




Fig. 6. Micro-angiogram of normal adrenal gland in 7 year-old hild. The capsular supply of the *cortex consists in sprays of the adult type* 40.

## Discussion

For technical reasons autopsy specimens from the fetuses and newborn were in jected from the aorta while the surgical specimens from the adults were filled from the venous side; to judge from the micro-angiographic and histologic examinations, the vascular pattern of the adrenal glands was similar for the two differently injected, series which may thus be regarded as com parable so far as the vasculature is con cerned (Figs. 1–6)

It was seen from the study that the vascular pattern of the adrenal gland *underwent a gradual change during the* development of the organ. At the beginning of the second trimester of pregnancy there was a central arterial supply with centrifugal vascular ramification, which was gradually replaced by a capsular supply with centripetal branching This change in pattern during development would seem to

Fig. 3. Micro-angiogram of fetal adrenal gland (fetal weight 1760 g age bout 34 weeks) The capsular arteries are now more numerous. 16. Fig 4 Micro-angiogram of fetal adrenal gland (fetal weight 2575 g, age bout 36 weeks). The capsular arteries are predominant. 16 Fig 5 (A) Micro-angiogram of adrenal gland from newborn (fetal weight 3645 g age 40 weeks). Detail of cortex with capsular plexus, showing the cortical arteries that end at arious levels and the medullary arterial branches. 40. (B) Histologic section of the region in Fig 5 A, with arteries penetrating the capsule *Hematoxylin and eosin* 40

occur parallel with the differentiation of the cortical zones and involution of the fetal cortex. It becomes of the adult type only after formation of the zona glomerulosa fasciculata and reticularis

It has long been known that the adult adrenal gland in man and several mammals has a centripetal arterial supply (man [6] dog [4] rat [8]) but that this develops gradually with the differentiation of the cortex would seem not to have been observed previously While the significance of this vascular re-construction is not apparent from this study it is probably due to a change in the general structure of the adrenal gland during fetal and neonatal life Of this function little is known [2] but the vascular alteration would point to a functional change covering a fairly long period. It is evident from the micro-angiograms (e.g. Fig 5A) that in some specimens the capsular arteries gave off branches to various levels of the cortex and are thus terminal arteries. This phenomenon has been described by Harrison [5] in the rabbit. To confirm the presence of such terminal arteries in the present series it would be necessary to *examine* serial sections, and this is now being done

The venous micro-angiographs of the surgical specimens verify that the organ has few veins which arise from confluent medullary veins these in turn originating from the confluence of the cortical sinusoids. These were filled from arteries given off at right angles from a capsular plexus; filling of the veins did not, however visualize the wider adrenal arteries.


## Summary

A micro-angiographic and histologic study has been made of adrenal glands from 20 autopsy specimens from fetuses and newborn up to 6 weeks of age and 13 normal specimens from mature glands. It was found that the vascular pattern of the adrenals changes with the progressive differentiation of the cortex


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# Pseudo Vitamin D Deficiency Rickets

## *Report of Four New Cases*

*by* J W STOOP M. J C. SCHRAAGEN and H A. W M. TIDDENS

In 1961 Prader *et al.* [1] described two patients with the clinical and biochemical features of rickets due to vitamin D deficiency occurring in spite of the administration of normal amounts of vitamin D Healing did not occur until after administration of exceedingly large amounts of vitamin D and subsequently these patients required a daily maintenance dose of 30 000-60,000 U Although vitamin D requirements in these cases were greatly increased, the clinical and biochemical aspects so closely resembled those observed in rickets due to vitamin D deficiency that Prader *et al.* rejected the diagnosis of conventional primary vitamin D-refractory rickets. On the basis of their findings they described the disease picture in these patients as *pseudo vitamin D deficiency rickets*

In the literature there are few other reports on this disease [4 5]. It is our impression however that this type of rickets is more common than the scarcity of publications suggest.

We present the clinical histories of four new patients in some detail adding the observation of catch-up growth under treatment to the disease picture

### *Patient 1*

We first saw Eddy when he was 30 months old. Pregnancy and parturition had been normal. Within the first year of life it had been noticed that the child s motor development was retarded. Due to environmental factors, no medical advice had been sought until the boy was 18 months old, t which time he could neither stand nor walk. Examination at that time had disclosed many healed or partly healed (spontaneous?) fractures: clavicular and humeral bones, right forearm, right femur The serum calcium level had been 6 mg/100 ml, and the serum alkaline phosphatase >120 U (King Armstrong).

Convulsions had occurred several time in the course of the next years, as had a few more fractures. The motor development had shown no progress. Eddy was reported to have received 1000 to 1500 U vitamin D daily from the age of 16 months. N abnormalities of habitus were reported in the maternal family The mother reported that Eddy father (whom we never saw) was of small stature and bow legged, as had been his father mother

At the age of 30 months Eddy was referred to the Wilhelmina Children Hospital for further diagnosis.

At *physical examination* we found his height to be only 76 cm; he showed marked hypotonia and could neither stand nor walk. H presented the typical symptoms of ric-

TABLE 1 *Review of the principal clinical and laboratory findings in our four patients*

| | Eddy | Barette | Jaap | Susanne |
|---|---|---|---|---|
| Onset 2–12 months | + | + | + | + |
| T tany | + | − | − | + |
| Spontaneous fractures | + | − | − | + |
| Dental defects | ? | ? | ? | ? |
| Height (after treatment) | About normal | Normal | Normal | Normal |
| Heredity | | + | + | − |
| Serum calcium ↓ | + | + | + | + |
| Serum phosphorus ↓ | | + | + | − |
| Serum phosphatase ↑ | + | + | + | + |
| Hyperchloraemia | − | + | + | − |
| Acidosis | − | + | + | + |
| Hyperaminoaciduria | ± | + | + | n d |
| Alpha-amino N in % of total N | 2 % | 3.5 % | 4.5 % | n.d. |
| Renal functions (concentrating capacity acid excretion, urea and creatinine clearances) | Normal | Normal | Normal | Normal |
| TRC-P | n.d. | 65 % | 92 % | d. |
| Liver functions | Normal | Normal | Normal | Normal |
| Steatorrhoea | | − | − | − |
| Total vitamin D requirement for saturation | $10^6$ U | 40 $10^6$ U | 14 $10^6$ U | 3 $10^6$ U |

kets, without other abnormalities. The skeletal *X-rays* were in accordance with a diagnosis of rickets, and in addition revealed multiple healed or partly healed fractures.

*Laboratory findings* Serum calcium 6 6 mg/100 ml, phosphorus 4 1 mg/100 ml; alkaline phosphatase 40 U (Bodansky) Serum sodium, potassium, chloride alkali reserve, urea and creatinine values were normal. Serum protein was 7 0 g/100 ml. Liver functions and renal functions were not impaired

As regards the urinary amino-acid excretion: the alpha-amino N was 2% of the total N which is a high but normal value. Protein and reducing substances were not found in the urine The urinary calcium excretion was 0.3 mg/kg/24 hours. No cystine crystals were found in the cornea. There was no steatorrhoea.

*Diagnosis and course.* Since the renal functions were normal, the rickets could not be interpreted as concomitant to a chronic renal affection. There were no clues to suggest a tubulopathy The low serum phosphorus level excluded hypoparathyroidism (Table 1)

The blood chemistry was compatible with rickets due to vitamin D deficiency but the patient's history revealed that he had received normal quantities of vitamin D for some considerable time.

In view of these facts we believed that this was a case of pseud vitamin D deficiency rickets of the type described by Prader et al. [1] The further course was in accordance with this suggestion.

High-dosage vitamin D medication, increasing from 10,000 to 100 000 U daily led to normalization of serum calcium, phosphorus and phosphatase values and urinary calcium excretion in about 46 days, i.e. after administration of a total of two million U vitamin D (Fig 1) At that time the urinary alpha-amino N had diminished to 1% of the total N The rachitic skeletal changes disappeared Catch up growth occurred, and for nine months the growth rate was considerably above the average for age (Fig 2). M tor development started quickly; Eddy spontaneously stood and could soon walk without support A drastic reduction of the vitamin D dosage was followed after some

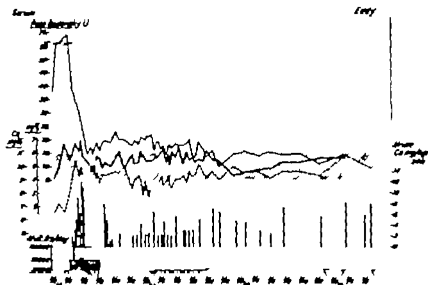


Fig. 1 Biochemical data on patient 1 in relation to amount of vitamin D administered

considerable time by recurrence of hypocalcaemia and hyperphosphatasaemia.

A perfect balance was struck with a maintenance dose of 21 000 U vitamin D daily (Fig. 1).

The growth rat is now in accordance with the average for age (Fig. 2). Cerebral development is slightly retarded.

## *Patient 2*

Suzette was 17 months old when admitted to our hospital.

Pregnancy and parturition had been normal and no neonatal difficulties had occurred. She had not been ill and had received an adequat diet including 600 U vitamin $D_2$

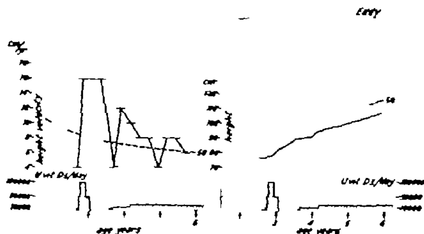


Fig. 2 Growth curve and growth rate curve of patient 1

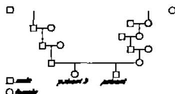


Fig 3. Pedigree of patients and 2.

daily her weight gain had always been sufficient. Mental development had been undisturbed but motor development had shown marked retardation. she had made no attempt t sit up and in fact had found it difficult to raise her head.

The paternal and maternal families are in good health. As Fig 3 shows, Suzett s parents are consanguineous. The father is of small stature.

At *physical examination* we found Suzette to be in good nutritional condition, weighing 10.6 kg for a height of 77 cm. The skull was square; the large fontanel measured 2 2 cm and had soft bony edges. The girl showed a rachiti rosary and thick wrists and ankles.

There was general muscular hypotonia, but no other bnormalities. *X-rays* of the wrists, ankles and knees showed unmistakable rickets.

*Laboratory findings.* Serum calcium 6.2 mg/100 ml, phosphorus 3.3 mg/100 ml, alkaline phosphatase 49 U (Bodansky) Serum sodium, potassium, urea and creatinine values were normal. The serum chloride level on one occasion was 120 mEq/l; subsequent determinations yielded values of about 110 mEq/l. The alkali reserve was initially slightly diminished to 19 mEq/l, but subsequent determinations always yielded normal values. Serum protein was 6.6 g/100 ml.

Liver functions were undisturbed. Renal urea and creatinine clearances, acid excretion (pH fasting urine: 5 0) and concentrating capacity were all normal. The phosphorus reabsorption coefficient was 85, which is just within normal limits.

There was aspecific generalized renal aminoaciduria, the alpha-amino N amounted to 4.5% of the total N Protein and reducing substances were not found in the urine. The urinary calcium excretion was 0.5 mg/kg/24 hours. No cystine crystals were observed in the cornea. There was no steatorrhoea (Table 1)

*Diagnosis and course.* For reasons similar to those pplied t patient 1 it was considered likely that this was a case of pseudo vitamin D deficiency rickets.

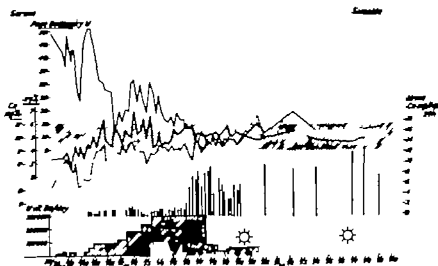
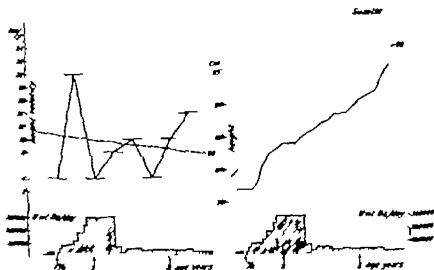


Fig 4. Biochemical data on patient in relation to amount of vitamin D administered.


Fig. 5. Growth curve and growth rate curve of patient 2.

High-dosage vitamin D medication normalized blood chemistry and urinary calcium excretion in bout 10 months; t that time the itamin D administration totalled bout 40 million U (Fig. 4). The aminoaciduria disappeared.

The skeletal changes disappeared and pronounced catch-up growth occurred (Fig. 5). Motor development was accelerated too. After recovery hypercalcuria on two occasions (following frequent exposure to sun light) necessitated red ction of the main tenance dose of 60,000 U vitamin D daily. A perfect balance was struck t da ly maintenance dosage of 30 000 U vitamin $D_2$.

## *Patient 3*

Jaap is younger brother f patient 2. Pregnancy parturition and neonatal period were uneventf l. N illness occurred during the first year f lif. He received a well balanced diet which from the onset con tained normal amount f itamin D (from age 5 months on ex as much s 1000 U daily).

When 6 months old he could roll himself bout but later this became more difficult H could not raise his head nd did not pull himself up.

*Physical examinati n* when he was 11 months old disclosed unequivocal rickets with caput quadratum, craniotabes, rachitic rosary and thick wrists and ankles. There was


Fig. 6. Wrist X-ra of patient 3 at the age of 6 months.



Fig. 7. Wrist X ray of patient 3 at the age of 11 months.

general muscular hypotonia, but no other abnormalities.

The *X-rays* epitomized rickets (Figs. 6 and 7).

*Laboratory findings.* In view of his sister's illness, Jaap was regularly submitted to determinations of serum calcium phosphorus and alkaline phosphatase values during the first year of life. An increase in alkaline phosphatase became visible at the age of 5 months; three months later this increase was much more marked, and calcium and phosphorus levels had fallen below normal (Fig. 8). At the age of 11 months, serum calcium was 8.6 mg/100 ml, phosphorus 3.0 mg/100 ml and alkaline phosphatase 45 U (Bodansky). Serum protein was 6.0 g/100 ml.

At that time there was a calciuria of 1.2 mg/kg/24 hours, which later diminished to 0.3 mg/kg/24 hours. Serum sodium, potassium, urea and creatinine values were normal. The serum chloride concentration was 110 mEq/l.

There was a mild metabolic acidosis with a blood pH of 7.31 and a standard bicarbonate value of 16. After acid administration (100 mg $NH_4Cl$ per kg) the urinary pH fell

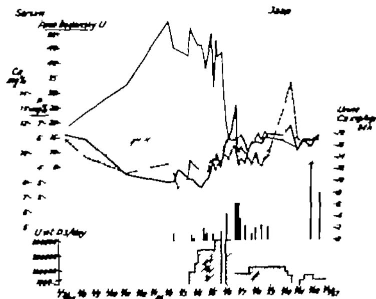




Fig. 8. Biochemical data on patient 3 in relation to amount of vitamin D administered.

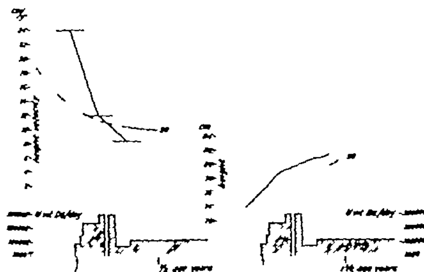


Fig. 9 Growth curve and growth rat curve on patient 3

to 5.0. Renal urea and creatinine learances and concentrating capacity were normal

The phosphorus reabsorption coefficient as 92, which is entirely normal. There was aspecific generalized renal ammoaciduria. the alpha-amino N amounted to 4.5% of the total N

N cystine crystals were observed in the cornea. Liver functions were not disturbed and there was no steatorrhoea (T ble 1).

*Diagnosis nd course.* On the basis of family history past history and clinical as well as biochemical features, t was consid ered certain that the boy suffered from pseudo vitamin D deficiency rickets.

In view of the parental consanguinity recessi autosomal hereditary transmission is probable The boy was treated with large doses of vitamin D orally which normalized blood chemistry and calcium in bout 11 weeks, at which time total of about 14 million U vitamin D had been given (Fig 8) The ammoaciduria and skeletal hanges disappeared.

Catch-up growth occurred (Fig 9) and motor dev lopment quickly started. Initially the boy seemed to require the very large maintenance dose of 120,000 U vitamin D daily He was discharged at this dosage

Six weeks later however he developed symptoms of vitamin D intoxication, until a perfect balance was struck at a daily dosage of 30 000 U

### *Patient 4*

Suzanne was admitted for observation t the age of 10 months, with a 14-day history f several brief convulsions daily sometimes accompanied by pronounced cyanosis. Preg nancy and parturition had been unev entful. She received a full-value diet and, from the 5th month of life on, 800 U vitamin D daily Paternal and maternal families were in good health.

At *physical examination* we found Suzanne to be a hypotonic Indonesian girl in good nutritional condition. The fontanel was open good three fingers A rosary was discer nible and the wrists showed unmistakable thickening The Chvostek reflex could not be elicited.

*X-rays* disclosed florid rickets and a healing spontaneous fracture of the right ulna.

th a diagnosis of vitamin D deficiency rkets.

*chemical differences* are unmistak e. *Hypophosphataemia* is a predominant ature of primary vitamin D-refractory ckets, the serum phosphorus value being lly less than 3 mg/100 ml, and perma nt normalization being difficult In eudo vitamin D deficiency rickets, the rum phosphorus concentration is either ightly diminished or completely normal 1 and 4)

*Hypocalcaemia* is the central feature in of rickets. The calcium concentra on is invariably below 8 mg/100 ml, and etany repeatedly occurs. Spontaneous fractures also are observed. In no case of prim ry vitamin D-refractory rickets has tetany been described [6]: the calcium concen tration is in fact often completely normal.

In both types of rickets the *alkaline phosphatase* is increased, very high values ndeed can be attained in pseudo vitamin D deficiency rickets. Adequate therapy ensures complete normalization of the blood chemistry in patients with pseudo vitamin D deficiency rickets. In primary vitamin D-refractory rickets, however it can be exceedingly difficult to normalize phosphorus values completely Such attempts always entail a risk of complication in the form of hypercalcaemia as a mani festation of vitamin D intoxication

*Aminoaciduria* and mild *acidosis* com bined with mild hyperchloraemia are observed in pseud vitamin D deficiency rickets [1] but r in primary vitamin D-refractory ri ts [6]. These hanges disappear in course of treatment The aminoaciduria is observed also in ordinary rickets due to vitamin D deficiency [7], as is the mild acidosis [8, 9] in some cases.

The *growth reta d* which invariably accompanies pseud n D deficiency rickets, is corrected r treatment Growth velocity reach level (catch up growth) No catch up th is seen in primary vitamin D-refra ry ri kets, but growth can be normal if t tment is star ted sufficiently early [1 ] thus ha been confirmed by Fanconi [11] Finally we must mention that both types of rickets are *hereditary* Primary vitamin D-refrac tory rickets is characterized by dominant X-chromosomal or dominant autosomal transmission while the transmission of pseudo vitamin D deficiency rickets is possibly of the recessive autosomal type ([1]: patients 2 and 3)

The above makes it clear that we are dealing with two different disease pictures.

The disturbance underlying pseudo vita min D deficiency rickets has not been iden tified with certainty Jonxis [4] assumes that his patients—who required 2000–3000 U vitamin D daily and developed rickets when given smaller doses—were suffering from reduced sensitivity to vita min D Soriano *et al.* [8] corroborated this assumption in that they ruled out a disturbance in vitamin D absorption in their patients. It seems conceivabl that our patients suffered from a much more severe degree of th same disturbance [1 ]

Further investigation will have to settle this question.

## Summary

Report of fou new cases f Pseud vitamin D deficiency ricket Clinical histories re presented in some detail. Differences with primary vitamin D refrac tory rickets are discussed.

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# Familial Protein Intolerance with Deficient Transport of Basic Amino Acids

## An Analysis of 10 Patients

by M. KEKOMÄKI, J. K. VISAKORPI, J. PERHEENTUPA and L. SAXÉN

Familial protein intolerance with deficient transport of basic amino acids (PI) is a syndrome of aversion to protein-rich nutrients with hyperammonemia and deficient rise in plasma urea levels following protein intake, failure of growth, hepatomegaly, characteristic amino aciduria, and periods of diarrhea and vomiting in infancy. It was described by us in three children in 1965 [22]. Since then, we have seen seven other Finnish and Lapp children with this syndrome. Here we define the clinical features of this disorder and present further investigations on its biochemistry and a preliminary evaluation of its treatment with arginine.

## Presentation of Patients

### Clinical characteristics

*Family and birth histories.* There were three pairs of siblings among our 10 patients (Table 1). The parents were second cousins in one family (the only Lapp patients 4 and 5). In a preliminary work-up of 3–6 generations of the genealogies none of the other parents has been found to be relatives. Urinary amino acid analyses from all (14) of the parents and from 15 of the 19 living asymptomatic siblings revealed increased lysine and cystine excretion in one mother and her daughter (the Lapp family).

Threatened abortion had complicated the pregnancy in cases 1, 2, and 9 (in the 1st trimester), and 8 (in the 3rd trimester). Gestation had proceeded normally in the other cases. The neonatal period was uneventful, except in cases 3 and 9, in which a slightly peculiar facies with macroglossia, and signs of thrombocytopenia, respectively, were noted. Hepatosplenomegaly was also noticed at that time in both of these patients.

*Symptoms of protein intolerance.* All ten patients tolerated breast milk without symptoms. A few months after weaning vomiting and diarrhea appeared, and the patients lost appetite and ceased to thrive. Two of the patients (3 and 9) were under our continuous observation during this period. Patient 3 tolerated ½-strength cow's milk well but became ill on ⅔-strength milk. Patient 9 apparently did not tolerate even ⅓-strength cow's milk during her first year of life. Once able to select their food by the 2nd year all patients systematically rejected cow's milk, as well as all meat, fish, liver and eggs. Some

This study was supported by the Foundation for Pediatric Research in Finland.

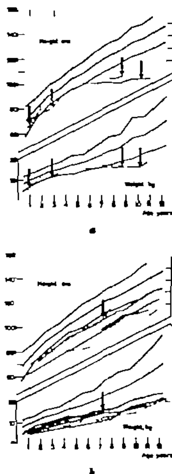


Fig. 1. Growth curves of 9 children with familial protein intolerance, girls *a*, boys *b*. The shaded area shows the 3–97 percentile range of Finnish children [20]. The black arrows indicate the beginning of arginine-HCl supplementation.

of them did not eat peas. Their diet consisted mainly of grain products, vegetables, fat, and juices. On this self-restricted diet the tendency to diarrhea subsided. All continued to have occasional vomiting.

*Physical findings.* All the patients were markedly retarded in growth, being at or below the 3rd percentile for height when seen by us (Fig. 1). The weights were slightly below the average for the height (mean: 95 per cent, range 83–114 per cent of the average; [20]). Reduced muscle mass and markedly subnormal physical fitness were recorded in all of them. Two of the patients had had two fractures each, reportedly from a minimal trauma. These fractures of tibia and femur were apparently slow in healing (refracturing after 6 months in each case). All had enlarged liver observed at at least some stage of the disease. Some had enlarged spleen (Table 1). An anterior polar cataract was found in patient 1. Nails and hair were seemingly normal in all.

### *Laboratory findings*

The relevant data from routine laboratory work-up are given in Table 2. The findings reflecting nitrogen metabolism were normal: plasma protein content with its electro- and immunoelectrophoretic pattern, and plasma amino nitrogen and blood ammonia concentrations in the fasting state. Infant patients may present elevated ammonia levels when fasted only 5 hrs. Fasting plasma urea concentration was, on average, distinctly lower than the median (12 mg per 100 ml) obtained from children hospitalized here, and levels as low as 4 and 5 mg per 100 ml were frequently found. Renal excretion of creatinine was less than expected for height. The creatine/creatinine ratio did not differ from the normal. The daily excretion of guanidinoacetic acid was also normal in the two cases studied.

Intestinal absorption was studied during the diarrheal period in three of the patients (Table 2). In patient 3, generalized malabsorption was evident. In patient 9 only the FIGLU test was positive, while in patient 2 the absorption tests were normal. In all the other patients these tests were performed after infancy with normal results except for a slightly pathologic FIGLU test in cases 1 and 8. The histologic picture of the mucosa of the small intestine, biopsied during the diarrheal period in cases 3 and 9, and during the non-diarrheal period in case 6, was that of normal villous structure and epithelial pattern, but with round cell infiltration of the lamina propria.

Liver function tests did not reveal any consistent abnormality (Table 2).

TABLE 1 *The clinical characteristics[a] of patients[b] with the familial protein intolerance syndrome.*

| Case No. | Sex | N of siblings | Birth wt. (g) and length (cm) | Age at weaning (months) | G-I symptoms after weaning | Protein aversion | Sporadic symptoms after infancy: Vomiting | Sporadic symptoms after infancy: Abd. pains | Growth retardation | Reduced physical fitness | Presenting signs: Hepato-splenomegaly[c] (liver) | Presenting signs: Hepato-splenomegaly[c] (spleen) | Intelligence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 1 | 2890 | 3 | + | + | + | + | + | + | 4 | — | High |
| 2 | M | Brother of N 1 | 2680 | 9 | + | + | + | + | + | + | 5 | 3 | Normal |
| 3 | M | 1 | 3870 (50) | 12 | + | + | + | ? | + | + | 2 | 3 | Normal? |
| 4 | M | 3 | 3000 | 12 | + | + | + | + | + | + | 8 | 2 | Normal |
| 5 | M | Brother of N 3 | 4000 | 4 | + | + | + | + | + | + | 6 | — | Normal |
| 6 | F | 4 | 2100 (B-triplet) | 10 | + | + | + | + | + | + | —[d] | —[d] | Mildly subnormal |
| 7 | F | Sister of N 6 | 3350 | 12 | + | + | + | + | + | + | 4 | — | Normal |
| 8 | F | 8 | 2850 | 8 | + | + | + | + | + | + | 4 | — | Normal |
| 9 | F | 4 | 2790 (50) | 12 | + | + | + | ? | + | + | 5 | 2 | Normal? |
| 10 | F | 3 | 2600 (B-twin) | 7 | + | + | + | + | + | + | 4 | — | Moderately subnormal |

[a] The clinical examination was performed at the age given in Table 2.
[b] Cases 1–2 have been reported previously [22].
[c] Liver and spleen size expressed in centimeters below costal margin.
[d] The liver and spleen palpable at 9 months, and only liver at 7 years of age.

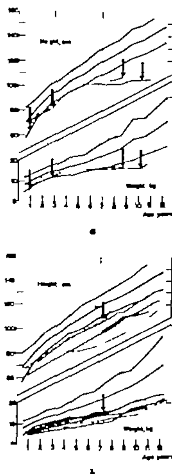


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The appearance of ossification centers was, on average delayed (Table 2). In the two patients with histories of fractures, definite cortical thinning was observed in x ray studies. The absence of signs of rickets in the bones was consistent with normality of serum alkaline phosphatase activity and of calcium and inorganic phosphate concentrations in all cases.

*Liver histology*

Liver tissue was obtained at laparotomy from patients 9 and 10 and with a needle from patient 4.

All three samples showed a fairly well preserved, normal liver architecture with only slight irregularities in patient 10. The variations in the parenchymal cells were within normal limits, and their granulation suggested a normal glycogen content except for some peripheral hepatocytes in the same patient, who had hydropic cytoplasm indicative of increased glycogen (Fig 2B) In cases 4 and 10 the richly granulated cells in the lobular periphery contained vacuolated nuclei which appeared in routine sections as large empty vesicles pressing the nucleolus towards the nuclear periphery The glycogen stain according to Best was positive and visualized as shrunken droplet in the nucleus (Fig. 2B insert)

The main abnormal feature in all specimens was found in the portal tract, where a cellular infiltrate consisting of large lymphocytes and macrophages was seen. In case 9 fibrosis was more profound and indicative of incipient diffuse cirrhosis (Fig. 2A). The conclusion was drawn that the changes may represent different stages of one and the same process, beginning as portal inflammation seen in the youngest of the patients with a gradual transition into diffuse cirrhosis seen in the oldest of them.

## Special Studies

### Methods

*Urine and plasma* -amino nitrogen (amino-N) were determined with a ninhydrin method [33]; urea and total N were measured by the urease-phenol-alkaline hypochlorite method [27], and by the micro-Kjeldahl technique [23], respectively The renal amino acid excretion pattern was studied by a high-voltage electrophoresis (HVE) technique [31]. For quantitative amino acid analysis, plasma extracts [29] and urines were stored at −20° (up to 6 months); the analysis was performed with an automatic ion-exchange procedure [20]. From the results the mean clearances for the 6- or 24 hr collection period were calculated.

The capacity for urea synthesis was studied by series of amino-N loads, in which per kg of body weight 6.6 mmoles of l-alanine was given 1) alone 2) with 1 1 mmoles of l-arginine- and 3) 1 1 mmoles of l-lysine-HCl. The constant infusions of 5 per cent aqueous solution lasted 90 minutes. Blood ammonia [14], urea, and amino-N were followed, and on some occasions CSF ammonia was determined. The times to be referred to are times elapsed from the start of the infusion.

*Seven control patients (from 3 months to 6 years old)* with asymptomatic cystinuria (1 patient) untreated rickets (1) granulomatous inflammation of liver and intestine with generalized amino aciduria and intestinal malabsorption (1) narcolepsy (1), and with primary gluten-induced malabsorption syndrome (3 patients) were subjected to the *lysine-alanine load only*

### Results

*Plasma amino acid concentrations* Compared with normal values for the same age group (Table 3) from Scriver & Davies [26] and Dickinson et al [6] the patients had reduced concentrations of lysine, arginine leucine and tyrosine whereas alanine citrulline and serine were present in some excess.

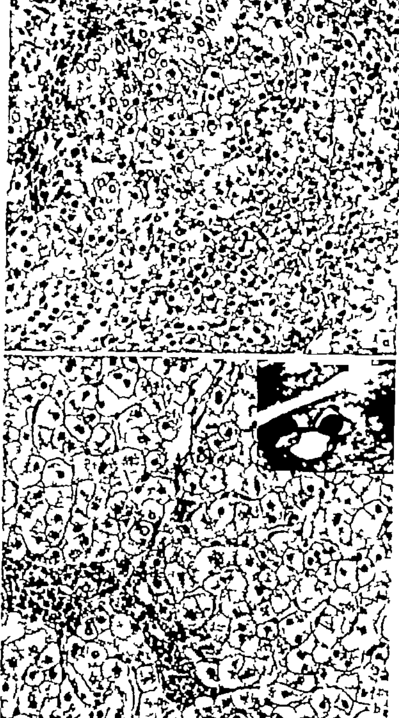



Fig. 2. Micrographs of the live specimen from patient 10.

*a.* A section of liver lobe showing the central vein in the lower right part and the portal tract in the upper left corner. The latter shows fibrous and cellular infiltration consisting of lymphocytes and macrophages. Hepatocytes in the peripheral part of the lobe are richly vacuolated and show large nuclei with an unstainable central portion. H & E 250

*b.* Peripheral hepatocytes with large hydropic cytoplasm and some vacuolated nuclei. Not the inflammatory reaction in the portal tract 400.

*Insert.* A parenchymal cell stained with the Best reaction for glycogen showing stained droplet in the nucleus. 900.

TABLE 3 *Plasma amino acid concentrations (mg/100 ml) in three patients (1 4 and two determinations from patient 5) with familial protein intol nce*

| Amino acid | Patients with familial protein intolerance (mean and range) | Normal children from Dickinson *et al* [6] (mean ± S.D.) | Normal children from Se er & Da es [26] (range) |
|---|---|---|---|
| Alanine | 5.57 (2.06–8 21) | 2.04 ± 0 49 | 1 ... 71 |
| Arginine | 0.34 (0.26–0.40) | 0 94 ± 0 09 | 0 40–1 49 |
| Aspartic acid | 0.11 (0 03–0 13) | 0 11 ± 0.05 | 0.03–0... |
| Asparagine + glutamine | 1.05 (0 95–1.21)[a] | 11 16 ± 2.04 | 0.84 6 91 |
| Citrulline | 1 03 (0.75–1 60) | 0.28 ± 0 09 | — |
| Cystine | 1.26 (1.20–1.36) | 1 47 ± 0 32 | — |
| Glutamic acid | 4 09 (1.58–6.36)[a] | 0 70 ± 0.37 | 0.24–3.66 |
| Glycine | 2.67 (1 71–3.07) | 2.83 ± 0.5 | 0.88–1.67 |
| Histidine | 1.16 (0.89–1.83) | 1.10 ± 0.25 | 0 37 1 31 |
| Isoleucine | 0.37 (0.29–0.50) | 0.6 ± 0.11 | 0 26–1 10 |
| Leucine | 0.64 (0.56–0 74) | 0.95 ± 0.23 | 0 73–2.33 |
| Lysine | 0.81 (0.58–1 10) | 2.93 ± 0 67 | 1 03–2.20 |
| Methionine | 0.33 (0.26–0.40) | 0 44 ± 0.1 | 0 16–0.33 |
| Phenylalanine | 0.47 (0.42–0 60) | 1.30 ± 0.23 | 0 4 1 01 |
| Proline | 2.40 (1 71 3 07) | 2.13 ± 0 36 | 0.89–1 94 |
| Serine | 2.12 (1 69–...4) | 1.73 ± 0.36 | 0.8 1 16 |
| Threonine | 1 11 (0.85–1 64) | ...89 ± 0.25 | 0.80–1.13 |
| Tyrosine | 0.48 (0.30–0.70) | 1.36 ± 0.30 | 0.56–1.29 |
| Valine | 1 42 (1.19–1 60) | 1.80 ± 0 48 | 1 49–3 31 |

[a] Glutamic acid/glutamine + asparagine ratio is inverted, presumably due to the long (about 4 months) storage prior t chromatography of the amino acid extract of plasma

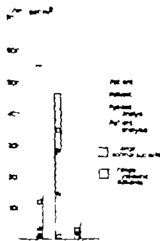


Fig. 3. The renal clearance of arginine lysine and cystine in 3 children with familial protein intolerance with values for homozygous cystinurics and normal subjects [2].

*Urinary excretion of amino acids* In the fasting state the amino-N/total N (or amino-N/urea-N) ratio was found to vary between 3 and 8 per cent HVE revealed hyperlysinuria with otherwise normal pat

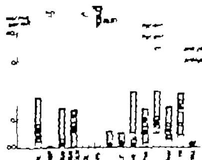


Fig. 4. The renal clearance of other amino acids, with values for normal subjects [2].

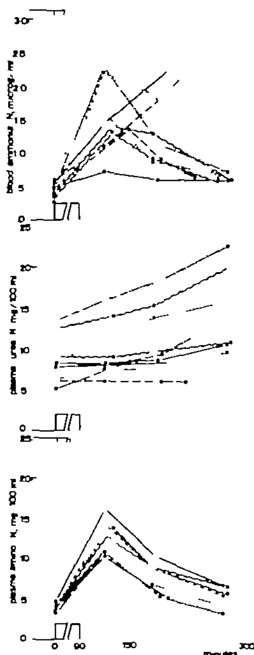



Fig. 5. Blood ammonia, plasma urea, and plasma amino-N levels after intravenous infusion of 6.6 mmoles of l-alanine/kg body weight in 9 patients with familial protein intolerance. For symbols see below.

| | | | |
|---|---|---|---|
| — — — — | 1 | ———— | 6 |
| ———— | 2 | ———— | 7 |
| ———— | 3 | ———— | 8 |
| ———— | 4 | ———— | 9 |
| ———— | 5 | — — | 10 |

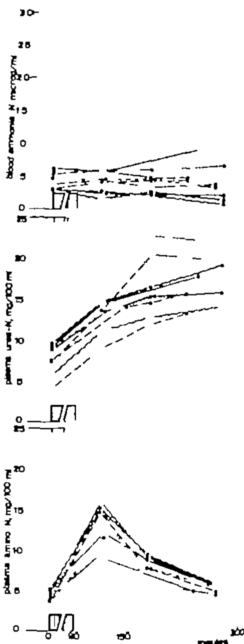



Fig. 6. Alanine infusion as in Fig. 5 with 1.1 mmoles of l-arginine-HCl/kg of body weight.

term. In cases 4 and 5 a cystine spot was also seen. Protein ingestion influenced the renal output of amino acids, the ratio rose and in HVE an increased excretion of

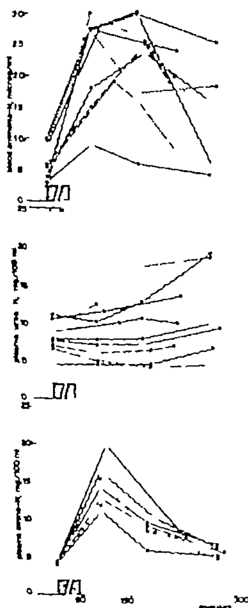


Fig. 7 Alanine infusion as in Fig. 6 with 1.1 mmoles of l-lysine-HCl/kg of body weight

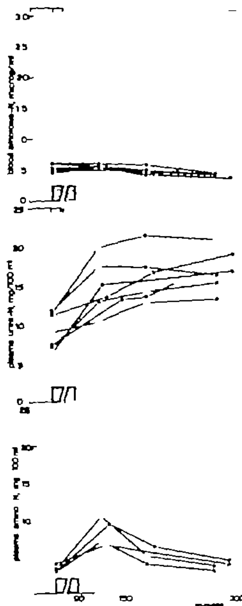


Fig. 8 Blood ammonia, plasma urea, and plasma serum K level in control patients after infusion of l-alanine l-lysine as in Fig. 7 Open circles patient with asymptomatic cystinuria.

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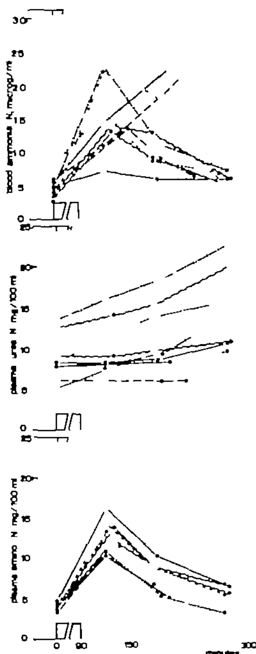



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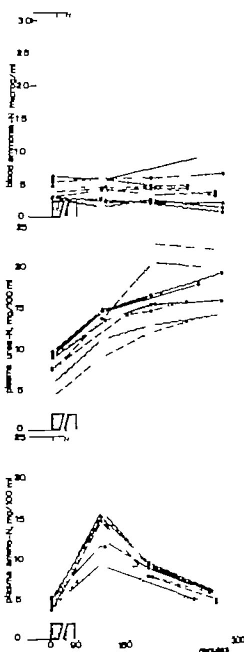



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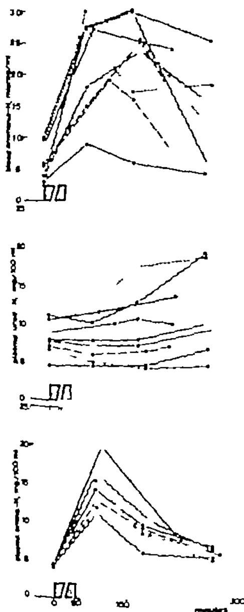


Fig 7 Alanine infusion as in Fig. 6 with 1 1 mmoles of l lysine-HCl/kg of body weight

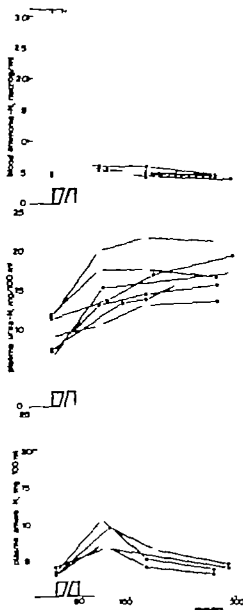


Fig. 8. Blood ammonia, plasma urea, and plasma amino-N level in control patients after infusion of l-alanine l-lysine as in Fig. 7 Open circles: patient with asymptomatic cystinuria.

arginine and to a lesser extent of all amino acids was observed.

*Renal clearance of amino acids* Comparison with measurements in homozygous cystinurics (Fig 3) and in normal subjects indicates that in PI there is an ccelerated clearance of lysine similar to that of cystinuria, while clearance of arginine is defi

nitely slower and of cystine only slightly if at all above normal. Because ammonia interfered in the chromatography the clearance of ornithine was not obtained. The clearances of other amino acids were normal (Fig 4)

*Amino-N loads* Figures 5–8 present blood ammonia, plasma urea and amino-N levels following the different loadings in the patients and controls. Table 4 gives the increments in plasma urea and amino-N at 2 hr

*Blood ammonia concentration* rose markedly in all PI patients (patient 8 excluded) after the alanine load, the peak being measured at 120–220 minutes. The mean increment was 1 0 μg/ml (range 0.20–1.80 μg/ml) CSF ammonia concentration was always lower than the simultaneous value of blood (0.9/3.5 0 4/1 1 and 0 1/0.5) Arginine abolished the hyperammonemic response In contrast, the hyperammonemia was clearly augmented in some patients when the lysine supplement was given. None of the controls had any increase in blood ammonia

The response to the alanine load in the *plasma urea concentration* was variable some patients showed no significant change in others a small and slow elevation was noted, while three of them (patients 2 3 and 7) exhibited a slow response with fairly normal peak increment The arginine supplement brought about a distinct rise in plasma urea concentration, of which, according to tests with arginine alone a part originated from the administered arginine itself (Table 4). The rest of the increase evidently reflected improvement of urea synthesis. The lysine supplement, on average diminished the rise in plasma urea concentration from that obtained with alanine alone but again with individual variations. In the controls there was a rapid elevation, in clear contrast to the PI patients

*Plasma amino-N concentration* exceeded the fasting level in all loading types at 120 minutes, the highest rise being found after the alanine-lysine load. The difference between controls (4 8 ± 1.8 mg per 100 ml) and patients (10.3 ± 2.8 mg per 100 ml) is statistically significant.

Both some of the patients and some of the controls occasionally complained of abdominal pains following the infusions. There was no clear difference in this reaction between different loads.

## Preliminary results of treatment with arginine supplement

The effect of oral arginine supplementation on urea synthesis was studied by comparing the courses of blood ammonia and serum urea concentrations in patient 4 after 1) 300 ml cow s milk and 2) the same supplement with 1.5 g arginine-HCl. The peak rises observed for ammonia N were 2.5 and 0 0 μg/ml and for urea-N −0 7 and 6.8 mg/100 ml at 5 hrs after the meal. As arginine was thus sufficiently absorbed to significantly improve urea synthesis even when given orally a trial of long term dietary supplementation was commenced.

At the time of this writing, the parents of the patients 1 through 5 have reported that for the last 16 months they have complied with the advice to get these children to take a total daily amount of 0 4–0.5 liter of cow s milk with 4 g of l-arginine-HCl

TABLE 4 *Plasma urea-N and α-amino-N concentration after the amino nitrogen load* *age*[a] *in patients with the familial protein intolerance and in control children. Incre ments (mmoles/l, mean ±S.D ) from fasting level 120 minutes after the start of the infusion*

| Load | Patients | | | Controls |
|---|---|---|---|---|
| | Alanine | Alanine + Arginine | Alanine + Lysine | Alanine + Lysine |
| Mmoles of amino-N per kg of body weight | 6.6 | 9.9[b] | 7 7 | |
| Urea-N | 0.9 ± 0.9 (9 | 4.1 ± 1 1 (9) | 0.1 ± 1.0 (9) | 3.9 ± 1.8 (7) |
| Amino-N | 6.5 ± 1 1 (7) | 6.4 ± 1.5 (7) | 7 4 ± 2.0 (7) | 3.4 + 1.3 (6) |
| Urea-N + amino-N | 7 4 ± 1.3 (7) | 10 4 ± 4 (7) | 7.3 ± 2.4 (7) | 7.3 + _1 (6) |

The loads (6.6 mmoles l-alanine alone or with 1 1 mmoles of l-arginine-HCl, or with 1 1 mmoles of l-lysine-HCl per kg of body weight) were given in aqueous solution as constant infusion lasting 90 minutes

[b] α-amino-N and guanidino-N

Number of subjects

added per liter During this period patients 1 and have started eating some fish and meat in addition to the milk dose Their rate of growth has unequivocally improved. The infant patient 3 is also reported to be as active as his elder (healthy) brother Patients 4 and 5 still find the milk unpalatable and have added little other protein-rich foods to their diet. This poorer success may in part result from a less supportive parental attitude The only side effect of the therapy has been the occurrence of somewhat loose stools for the first two months in the youngest patient 3.

## Discussion

Familial prot in intolerance with defi cient transport of basic amino cids is clearly established inborn error of met bolism, a definit entity in the miscel laneous group of disorders sharing th characteristic renal leakage of basic amino acids [ 3 9 10 11 1_ 13 4 28 32]. Two of our patient were found by ob taining dietary histories of the cysti nuries detected by screening of amino acids by HVE from urines sent to us from the pediatric hospitals of this country during the past six years.

The pattern of the familial occurrence in our series corroborates the probability of an autosomal recessive mode of trans mission. As the families live in different parts of the country there being no ten dency for accumulation of known ances tors in certain areas, we suppose that the mutant gene is not infrequent in our popu lation. As yet the families have not been adequately studied. The amino ciduria observed in a mother nd sister in an inbred family certainly is not a constant heterozygous feature

Several organs show an bnormal func tion in PI. In the kidney tubules the reabsorption is deficient for lysine to a lesser xtent for arginine and, possibly slightly for cystine Cystine reabsorption seems to be clearly better in the PI pa tients than in homozygous cystinuries of

any type [2 10 14 28]. This transport defect is apparently shared by the intestine [22]. The sum rate of translocation of a tracer dose of radioactive lysine and arginine from the extracellular space, corrected for urinary loss, was unaffected in PI [15]; the uptake of these amino acids by other tissues seems thus not to be impaired. The rate of urea production is definitely subnormal in spite of the normality of the activities of the associated enzymes as determined in liver tissue [16].

As yet, the interrelation between the defects of transport and urea synthesis has not been solved. We have postulated earlier [22] that the transport defect, together with the low intake, leads to lack of ornithine (and arginine). This theory is corroborated by two pieces of evidence: 1) urea production and ammonia detoxification are enhanced by administration of l-arginine (the former clearly more than expected from mere cleavage of the arginine moiety) and l-ornithine [22], and 2) plasma arginine levels are low in the patients. Although the effect of additional arginine on the ammonia accumulation after amino nitrogen loading is clear-cut, the high levels of plasma amino nitrogen after alanine-arginine infusion, as compared to the controls, indicate that arginine is not able to fully normalize the urea production in PI (Table 4). Furthermore, the plasma levels of ornithine and citrulline were normal (Table 3), which also calls for further investigation of amino-N elimination and liver urea synthesis. These are in progress along with quantitative studies on the intestinal absorption of the basic amino acids.

The occurrence of diarrhea and vomiting is clearly dependent on protein intake. Although recurrent vomiting is a feature common to inborn disorders of urea synthesis [1 18 25], no simple correlation has been observed between blood ammonia level and sickness [4]. This is consistent with the experience with our patients. Similarly, the mechanism of the diarrhea is unsolved. Besides the gastrointestinal symptoms, strong protein aversion is characteristic of PI. This feature is unknown in other disorders associated with hyperammonemia, except for a report of two patients with argininosuccinic aciduria [21]. This difference could conceivably be due to the fact that most of the patients with those other disorders have been severely mentally retarded.

Our patients showed severe retardation of growth without other clear-cut evidence of protein deficiency, such as hypoalbuminemia, in contrast to children suffering from ordinary protein malnutrition. Deficiency of arginine, the strongest stimulator of growth hormone release amongst the amino acids [19], might contribute to this pattern, the growth stimulus being relatively weak because of this specific defect, the protein available would better meet the other needs of the organism. Retardation of bone age is known to be associated with undernourishment [7] and also with growth hormone deficiency. The pathogenesis of the liver lesion is unknown. Protein malnutrition is a well recognized offender, but our patients lacked the characteristic histologic feature associated with it, fatty degeneration of the hepatocytes. Hepatomegaly has been reported to be associated with another specific defect of urea synthesis [5] in apparent absence of unbalanced nutrition.

## Summary

We describe ten children, Finnish and Lapp, suffering from the familial protein intolerance with deficient transport of base amino acids. This disease is probably transmitted by an autosomal recessive gene. The patients have hepatomegaly which may be congenital, present as infants with vomiting and diarrhea on weaning from breast feeding and later as dwarfs with a strong aversion to protein rich foods. They have a characteristic renal amino aciduria with excessive leakage of lysine and less of arginine. Their urea production is impaired: on protein administration ammonia accumulates in their blood, whereas there is a delay in the fall of plasma amino nitrogen and deficient rise in plasma urea concentration. Both arginine and ornithine improve urea synthesis. The preliminary results of treatment with a dietary arginine supplement are promising.

In a series of liver biopsies, the findings suggested a slowly progressing process leading to diffuse cirrhosis.



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# Enzymes of Urea Synthesis in Familial Protein Intolerance with Deficient Transport of Basic Amino Acids

by M. KEKOMÄKI NIELS C. R. RÄIHÄ and J PERHEENTUPA

Elevated blood ammonia concentration is characteristic of a number of inborn errors of metabolism [1 4, 13 21], in some of which a defect in one of the enzymes of urea synthesis has been demonstrated [14, 1 *4].

Perheentupa & Visakorpi [18] have described the syndrome of familial protein intolerance (PI) associated with a cystinuria-like deficient renal and intestinal (re-)absorption of basic amino acids. In this disease impaired elimination of ammonia is reflected by transient hyperammonemia and failure of the plasma urea concentration to rise after oral or intravenous amino nitrogen loads L-lysine administration further accentuates this response Since L-arginine or L-ornithine on the other hand, seem to normalize these findings, arginine deficiency has been suggested to be the cause of the impaired ammonia detoxification [18].

A specific defect in urea synthesis in the liver might be due to absence or kinetic abnormality of any of the enzymes involved, to substrate deficiency or to shortage of cofactors. In the present study the activity of the enzymes involved in urea synthesis was measured in the livers of * children suffering from PI, the kinetics of liver arginase was examined and the plasma concentrations of arginine and lysine were measured.

## Methods

*Determination of plasma arginine and lysine*

These were isolated by ion exchange [15] from samples taken after an overnight fast. Lysine was measured with ninhydrin, arginine with a Sakaguchi reaction [3].

*Enzyme studies*

Liver specimens obtained at laparotomy were immediately frozen in $CO_2$-ice A 10 per cent homogenat was prepared in cold 0.1 per cent cetyltrimethylammonium bromide All studies were completed within 48 hours of laparotomy

Carbamyl phosphat synthetase (EC 2.7.2.2), ornithine transcarbamylase (EC 2.1.3.3), argininosuccinase (EC 4.3.2.1), and arginase (EC 3.5.3.1) were determined by the techniques of Brown & Cohen [2]. The activity of the arginine synthetase system (= the overall reaction of Ratner [20]) was measured, using l-ureido-$^{14}$C-citrulline as substrate [19]. The protein content of the homogenate was estimated by the method of Lowry *et al.* [12]. Enzyme activities are expressed as the amount of enzyme which catalysed the formation of 1 $\mu$mole of product per hour per milligram of liver protein, under the assay conditions.

A part of the homogenate was diluted 1/20

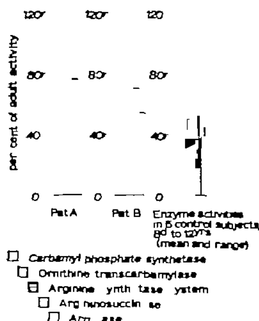



Fig. 1 Relative activities of carbamyl phosphate synthetase, ornithine transcarbamylase, the arginine synthetase system, argininosuccinase and arginase in the liver tissue of two patients with familial protein intolerance and in 5 control children.

The mean activity of 6 adult subjects, taken as 100 per cent reference was as follows: carbamyl phosphate synthetase, 1.9* µmoles product per hour per mg liver protein; ornithine transcarbamylase 66.7 µmoles product per hour per mg liver protein; arginine synthetase system, 0.60 µmoles product per hour per mg liver protein; argininosuccinase, 2.0 µmoles product per hour per mg liver protein; arginase 670 µmoles product per hour per mg liver protein.

with 0.2 *M* sodium glycinate buffer pH 9.5, and $MnCl_2$ was added to concentration of 0.01 *M* (to activate arginase). The initial velocities of the arginase reaction were determined at four arginine concentrations, ranging from 0.22 to 12.5 m*M*. The inhibition constants of l-lysine and l-ornithine were determined at the concentration of 6.25 m*M* of these amino acids. Incubation lasted 5 minutes in all experiments. The inhibition constant was calculated from the apparent kinetic constants in the absence and presence of inhibitor.

*The patients and control subjects*

The clinical findings in the two PI patients have been reported elsewhere [10]. In both patients, the diagnosis was confirmed by semi-quantitative amino acid analysis of the urine [27], and by intravenous amino acid loads [9, 10, 18].

The controls for plasma arginine and lysine concentration were 7 healthy children, from 6 to 12 years of age.

The controls in the enzyme studies were patients with presumably healthy livers, subjected to abdominal surgery for various reasons; 5 were children, from 8 days to 12 years of age and 6 adults, from 20 to 60 years of age.

## Results

*Plasma concentration of arginine and lysine*

Table I presents the findings in the two PI patients and the seven control children. The plasma arginine concentration was clearly lower in the patients than in the controls whereas no marked difference was found in the plasma lysine levels.

*Liver enzymes of urea synthesis*

The activities of five liver enzymes of urea synthesis are indicated in Fig. 1; the activities are expressed as percentage of the corresponding mean levels of the 6 adult controls. In the two patients the activity of the individual enzymes varied from 20 to 100 per cent. The activities of carbamyl phosphate synthetase, of the arginine synthetase system, and of arginase, however, fell within the range of the control children, whereas in both patients the ornithine transcarbamylase and argininosuccinase activities were clearly higher than in any of the controls.

*Kinetic properties of liver arginase*

The Lineweaver-Burk plot [11] of liver arginase for arginine is presented in Fig.

2a. The apparent $K_m$ values for the two patients were ..4 and 2.5 mM They fell within the range of the three control subjects studied (2.1 2.5 and 2.6 mM arginine).

The l-lysine and l-ornithine inhibition constants for the two patients were 0.7 and 0.9 mM (controls: 0 7 0.9 and 0 9 mM) for l lysine and 0.5 and 0 6 (controls. 0 5, 0.7 and 0.7) mM for l-ornithine respectively (Figs. b and 2c)

## Discussion

Descriptions have been presented of patients with hyperammonemia associated with diminished activity of ornithine transcarbamylase (in "hyperammonemia") [..1], argininosuccinate synthetase (in cit rullinuria ) [14], and argininosuccinase (in argininosuccinic aciduria ) [23] In citrullinuria, a kinetic variant of argininosuccinate synthetase has been found in cultured fibroblasts [24].

Since the hyperammonemia in PI can be prevented by administration of l-arginine or l-ornithine [10, 18], it might be due either to a kinetic abnormality of one of the enzymes involved in urea synthesis, or to a deficiency in arginine and ornithine The low plasma urea concentration found after intravenous amino acid loads could be further reduced by a dose of l-lysine which did not prevent plasma urea levels from rising in control patients [10]. As arginase is the only enzyme of urea synthesis known to be inhibited by l lysine [8] a kinetic study of liver arginase was of prime interest in this disorder The findings that liver arginase has an apparent $K_m$ for arginine and inhibition constants for l lysine and l-ornithine which do not differ

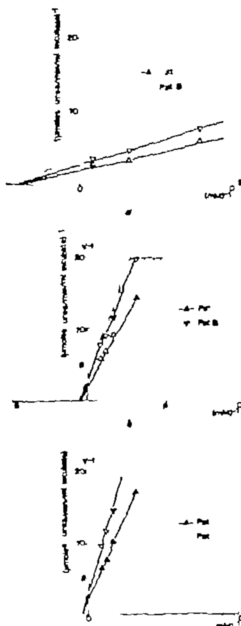


Fig. 2 ( ) Graphic estimation of the kinetic constant of liver arginase in two patients with familial protein intolerance The shaded area represents the range in 3 control children. (b) As but with the presence of 6 25 mM of l-lysine ( ) A a, but with the presence 1 6.25 mM of l-ornithine

TABLE 1 *Plasma arginine and lysine concentrations and their molar ratios in two patients with familial protein intolerance (PI) and in 7 healthy control children*

| | Plasma arginine concentration, µmoles/ml | Plasma lysine concentration, µmoles/ml | Molar ratio lysine/arginine |
|---|---|---|---|
| Patient A | 0.010 | 0.062 | 6.2 |
| Patient B | 0.015 | 0.066 | 4.4 |
| Control children, mean and range | 0.039 (0.031–0.055) | 0.080 (0.050–0.098) | 2.0 |

from those of the control subjects suggest that an abnormality of arginase is not the cause of the impaired ammonia detoxification through the urea cycle.

The activity of the other four enzymes of urea synthesis was only assayed in the presence of excess substrate: thus theoretically the possibility remained that one of the other enzymes involved is kinetically abnormal.

The presence of a distinctly subnormal concentration of arginine in the plasma of the two patients studied was confirmed. Since the plasma lysine decrease was not as marked, an increase in the molar ratio of lysine to arginine was evident (Table 1). This might be enough to cause some competitive inhibition of arginase and thus contribute to the impairment of urea synthesis.

It has been demonstrated that the amount of arginine is a potential limiting factor in urea synthesis. Arginine and ornithine reduce the toxicity of amino acids [7] and ammonia [10, 26] in animals. In some types of hyperammonemia following severe liver disease in man, arginine often has an ameliorating effect [5, 17].

The activities of the enzymes of urea synthesis show a direct correlation with the protein intake of the rat [22], whereas an arginine-free diet causes a rise in the activity of all of these enzymes except arginase [23]. It is interesting to find that in both of our protein-avoiding patients the activities of ornithine transcarbamylase and argininosuccinase were clearly higher than in any of the controls, whereas the activities of carbamyl phosphate synthetase, of arginine synthetase and of arginase were within the control range. This is consistent with the view that in this syndrome arginine deficiency contributes to the hyperammonemia.


## Summary

Urea synthesis was studied in the livers of two children suffering from the syndrome of familial protein intolerance with deficient transport of basic amino acids. The activity of arginase was within the control range in the presence of excess substrate. The apparent $K_m$ for l-arginine and the apparent inhibition constants for l-lysine and l-ornithine were also similar to those of the controls. With an excess of substrate the activity of carbamyl phosphate synthetase and of the arginine synthetase system were within the range of five control children, while the activity of ornithine transcarbamylase and of argininosuccinase were distinctly higher than in any of the controls. The plasma arginine concentration was low in both patients.

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# Studies of Urinary Tract Infections in Infancy and Childhood

## IX. Determination of E coli Antibodies by a Polyvalent Antigen

by HANS JÖRGEN ANDERSEN

Determinations of the antibody response to the infecting bacteria in patients with urinary tract infections have been found to be of value in classification of the severity of the infection, in control of the course, and as a diagnostic aid in doubtful cases [3, 4, 8, 19 22, 28, 29 30] However its use as a routine procedure has been limited by the practical difficulties of these determinations. The high specificity of the hemagglutination reaction, which has been employed by most investigators, necessitates the use of an antigen of the same bacterial serotype as the one infecting the patient. For this reason, antigens have mostly been prepared for titration of each patient s serum from his own urinary flora. This is a disadvantage from a practical point of view and is not possible in certain clinical situations where urinary tract infections are suspected, but no bacteria are found in the urine [3]

In an earlier paper [1] the results of a fruitless effort to use as standard antigen the common enterobacterial antigen, discovered by Kunin [13 14] were described. The purpose of this paper is to present experimental evidence for the possibility of using a polyvalent antigen (PA), consisting of 0-antigens from the E coli strains most commonly encountered in urinary tract infections. Secondly a material of patients with urinary tract infections in which antibody titres were determined with the PA as well as with the homologous antigens is presented.

This study was supported by Grants B46/B47 19x 743 from the Swedish Medical Research Council.

### Material and Methods

1 *Antisera* were prepared in rabbits against each of the following *E coli* 0-types: 01 02, 04 06, 07 08, 018, and 075. The bacterial strains were kindly supplied by Drs. F and I. Orskov The International Escherichia Centre, St tens Seruminstitut Copenhagen, Denmark. The bacteria were grown for ten hours on a synthetic medium, not immunogenic in rabbits, TY G 25 [12]. The bacterial suspensions were boiled for two hours and centrifuged at 3000 rpm for 30 minutes. The supernatant was kept in refrigerator until used. Eight rabbits were injected intravenously with one each of the antigen-suspensions. Six injections were given with five or six days intervals in doses of 0 2, 0.5 1.0 1.5, 2.0 and 2.0 ml. One week after the last injection the rabbits were sacrificed and the blood was collected. The serum was divided in small portions and stored in −70°C till used.

2 *Antigens* were prepared from each of the abovementioned *E coli* strains separ

ately The bacteria were grown on nutrient agar plates overnight, the growth was harvested and suspended in saline. The bacterial concentrations were adjusted to about 1200 million organisms per ml by comparison to a McFarland scale These suspensions were boiled for two hours and centrifuged for 30 minutes at 3000 rpm. The supernatants were used as antigens.

3. *Sensitization of red blood cells* Sheep red blood cells washed three times in saline were used as carriers of the bacterial antigens. 0.1 ml washed and packed blood cells were incubated for 30 minutes at 37°C with 5 ml of the antigen to be used. For sensitization with more than one antigen, equal amounts of antigen suspensions were added simultaneously to the blood cells. It should be observed that when more than one antigen is used for sensitization, the concentration of each single antigen is less than when they are used individually Thus, when eight antigens are mixed, as in most cases in this study the concentration of each antigen is reduced to 1 8 After incubation, the modified red blood cells were washed three times in saline and were finally diluted in saline to a concentration of 1%.

4 *Hemagglutination.* Aliquots of 0 1 ml of a twofold serial dilution of the serum specimen to be tested were placed in the cups of a perspex tray 0.1 ml of the 1% suspension of modified sheep red blood cells were added to each cup The perspex trays were incubated for three hours at 37°C and the results were read grossly after gentle shaking Two parallel determinations were always performed on each serum sample. The controls were uncoated sheep red blood cells in undiluted serum and coated sheep red blood cells in saline

5. *Hemagglutination inhibition.* These experiment were performed as described by Broberger & Perlmann [6].

6. *Patients* This material consisted of 29 patients, 21 girls and 8 boys aged 1 month to about 10 years, suffering from urinary tract infections, giving an elevated antibody titre against the homologous urinary flora and/or the PA.

Twenty-five of the patients were classified as cases of acute pyelonephritis according to the criteria described in detail in earlier studies [4 30], i.e. a history of frequent and burning micturitions or in the smaller children, of irritability vomiting, pallor and tenderness on palpation of the abdomen or loins, and the findings of fever elevated erythrocyt sedimentation rate, reduced renal concentrating capacity leucocyturia and bacteriuria.

Two patients were hospitalized for enuresis (Cases no. 2 and 12) Both of these had earlier suffered from acut pyelonephritis according to the above-mentioned criteria and were now found to have afebrile relapses with the enuresis as the only clinical symptom.

Case no. 24 had at the actual infection an afebrile relapse with frequent and burning micturitions. No. 26 had asymptomatic bacteriuria, detected on a follow up investigation. Both of these patients had earlier had overt acute pyelonephritis.

Less than 100,000 organisms per ml of urine was found in four of the patients of this material (Cases no. 7 17 24 and 28). They were included in this study as most other findings as well as their clinical symptoms were typical for acut urinary tract infections.

Sera from the patients were collected at different times during their illness. Antigens were prepared from the patients urinary strains, isolated before treatment was instituted The serological typings of these strains were performed in two laboratories, preliminary typing of the 0-antigens was done by Dr K. Lincoln, Department of Clinical Bacteriology University of Gothenburg. The definite typing of the 0- and H-antigens was performed by Drs. F and I Ørskov The International Escherichia Centre Copenhagen, Denmark.

Hemagglutination titrations on the patients' sera were performed with the homologous antigens as well as with the PA. The titrations with the two antigen-preparations were done in duplicate and were always performed simultaneously

TABLE 1. *Titre of rabbit-sera against corresponding* E. coli *O-antigens before first and after last antigen injection.*

The results are expressed as titration steps.

| Antiserum to | Titre before immunization | Titre after immunization |
|---|---|---|
| 01 | 2 | > 25 |
| 02 | 3 | 23 |
| 04 | < 1 | 23 |
| 06 | < 1 | 26 |
| 07 | < 1 | 23 |
| 08 | < 1 | 21 |
| 018 | < 1 | > 25 |
| 075 | 1 | 23 |

7 *Controls.* 133 children, 81 boys and 52 girls, aged from 17 days to 17½ years, were used as controls. They were all hospitalized for different non-infectious diseases, mainly psychosomatic and neurologic disorders. None of them had a history of previous urinary tract infections. The erythrocyte sedimentation rate was not elevated in any and none had fever. Asymptomatic bacteriuria was excluded by means of quantitative urinary cultures in all. Serum samples were collected as soon as possible after the admission. Two parallel determinations of the titre against the PA were performed on each serum sample.

## Results

### A. *Rabbit* E. coli *antisera*

Serum samples from each of the rabbits were titrated with the corresponding coli antigen before and after immunization of the animals. The results of these titrations are given in Table 1 from which it can be seen that three of the rabbits (1, 2 and 75) had very low titres, the rest no detectable reactions, before the immunization. In all animals a very marked increase in the titres was obtained during the immunization.

The possibility of cross reactions between the sera and the antigens included in this study was investigated by cross-matching all eight sera against each of the eight antigens. These results are presented in Table 2 from which it appears, that cross reactions were quite common but all giving much lower titres than the homologous reactions. Further the sera were tested with a crude preparation of the common enterobacterial antigen, prepared from a strain of 014. The titres against this were all very low.

Table 2 shows a comparison of the re

TABLE 2. *Results of hemagglutination titrations performed with the employed antisera cross-matched with the employed antigens and with a crude preparation of the common enterobacterial antigen (CA).*

The results are expressed as titration steps.

| Antiserum to | O-antigen no. 1 | 2 | 4 | 6 | 7 | 8 | 18 | 75 | CA |
|---|---|---|---|---|---|---|---|---|---|
| 01 | 25 | 6 | < 1 | 1 | 1 | 8 | 2 | < 1 | 1 |
| 02 | 5 | 23 | 2 | 2 | 3 | 6 | 2 | 1 | 1 |
| 04 | 2 | 3 | 23 | 2 | 5 | 7 | 7 | 3 | 2 |
| 06 | 4 | 4 | 2 | 26 | 2 | 5 | 3 | 4 | 1 |
| 07 | 4 | 5 | 3 | 3 | 23 | 5 | 4 | 4 | 3 |
| 08 | 3 | 4 | 2 | 2 | 3 | 21 | 3 | 3 | 1 |
| 018 | 3 | 4 | 7 | 1 | 2 | 6 | 23 | 2 | 2 |
| 075 | 6 | 5 | 2 | 2 | 3 | 6 | 3 | 23 | 2 |

TABLE 3. *Results of simultaneous determinations of the titres of the employed antisera tested with sheep red blood cells sensitized with the homologous antigens individually or with a mixture of all employed antigens*

The results are expressed as titration steps.

| Antiserum to | 0-antigen no. 1, 2, 4, 6, 7, 8, 18, 75 | 1 | 2 | 4 | 6 | 7 | 8 | 18 | 75 |
|---|---|---|---|---|---|---|---|---|---|
| 01 | 24 | 25 | | | | | | | |
| 02 | 23 | | 23 | | | | | | |
| 04 | 25 | | | 25 | | | | | |
| 06 | 26 | | | | 28 | | | | |
| 07 | 26 | | | | | 27 | | | |
| 08 | 21 | | | | | | 23 | | |
| 018 | 21 | | | | | | | 24 | |
| 075 | 23 | | | | | | | | 26 |

sults obtained in simultaneous titrations of the eight antisera with their corresponding antigen and with PA. The table illustrates the good correlation between the titres obtained with the two antigen preparations.

Table 4 presents the results found when hemagglutination with all eight antisera were performed with red blood cells, sensitized with five of the corresponding antigens. The results are compared to simultaneous titrations with the eight antigens individually. The very low titres of the antisera to *E. coli* 02, 06 and 08 against the mixed antigens demonstrate the specificity of the reaction. Several similar comparisons with varying numbers and combinations of the employed antigens were performed. The results were always in agreement with the example given in Table 4.

To further ascertain the specificity of

TABLE 4. *Results of simultaneous determinations of the titres of the employed antisera tested with sheep red blood cells sensitized with the homologous antigens individually or with a mixture of five of the employed antigens*

The results are expressed as titration steps.

| Antiserum to | 0-antigen no. 1, 4, 7, 18, 75 | 1 | 2 | 4 | 6 | 7 | 8 | 18 | 75 |
|---|---|---|---|---|---|---|---|---|---|
| 01 | 23 | 24 | | | | | | | |
| 02 | 3 | | 23 | | | | | | |
| 04 | 5 | | | 23 | | | | | |
| 06 | 2 | | | | 26 | | | | |
| 07 | 0 | | | | | 23 | | | |
| 08 | 3 | | | | | | 21 | | |
| 018 | 22 | | | | | | | 16 | |
| 075 | 21 | | | | | | | | 25 |

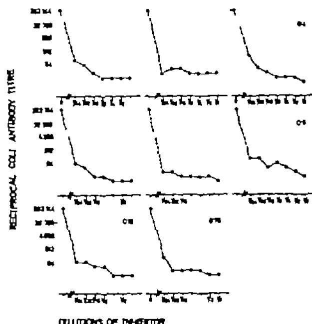



Fig. 1. Results of hemagglutination inhibition experiments on rabbit *E coli* antisera with the corresponding antigen as inhibitor and the PA as indicator

the reaction obtained with the antisera hemagglutination inhibition experiments were performed with each of these. Red blood cells, sensitized with PA, were used as indicator and the antigen, homologous to the tested serum, was used as inhibitor. The results for the eight antisera are illustrated in Fig. 1 which shows that all the antisera lost practically all their activity against PA by preincubation with their homologous antigens.

B *Control patients*

The titres found in the controls are illustrated in Fig. 2, which shows that the maximal titre in all age groups included in this study was $10^{-4}$.

From the distribution of the results it appeared likely that some correlation might exist between the height of the titre and the age of the controls. To find this possible correlation, the results were analyzed in a computer programmed for calculation of polynomial regression according to the least square method [18] and the following curvilinear regression was computed.

$$y = 2{,}667 - 5{,}901\,x + 14\,036\,x^2 - 4{,}853\,x^3 - 2{,}205\,x^4 + 0{,}981\,x^5$$

where $y = {}^{2}\log$ titre and $x = {}^{10}\log$ age (in months). The mean level given in Fig. 2 is based on this equation.

The deviation from the regression curve was found to be of normal distribution with a mean ~ 0 and 2 S.D. were calculated to be 1 9 titration step.

C *Patients*

The patients were divided into three groups with regard to the relationship between the antibody titres, measured by

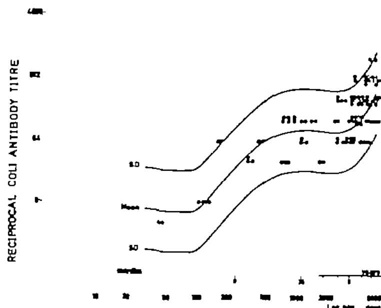



Fig. 2. Results of titrations with the PA in 133 controls. Open circles, girls; full circles, boys.

TABLE 5 *Sex, age, previous history and serological* E. coli *type in 18 patients belonging to group 1 (corresponding titres by means of homologous antigen and PA)*

| Case no. | Patient | Sex | Age (year/month) | Earlier urinary tract inf | *E coli* type |
|---|---|---|---|---|---|
| 1 | E. H. | F | 1 y 0 mo. | N | 01 H7 |
| 2 | A. B | F | 2 2 | Yes | 01::H7 |
| 3 | A. N | F | 3 5 | Yes | 01::H7 |
| 4 | S. R. | F | 9 3 | Yes | 01::H |
| 5 | T J | M | 0 6 | N | 02::H1 |
| 6 | N W | F | 0 5 | N | 02::H4 |
| 7 | A. A. | F | 5 0 | N | 02::H4 |
| 8 | K. N | F | 0 5 | No | 04::H5 |
| 9 | J B. | M | 0 6 | N | 04::H5 |
| *10* | *A. E.* | *F* | *0 10* | *N* | *06::H1* |
| 11 | K. F | F | 9 1 | Yes | 07::H– |
| 12 | M. E. | F | 3 11 | Yes | 07[b] |
| 13 | C. T | F | 6 7 | Yes | 018::H7 |
| 14 | I. H. | F | 0 5 | N | 018::H |
| 15 | S. L. | F | 5 5 | N | 018::H |
| 16 | A. S. | F | 8 6 | N | 018 H |
| 17 | M. O | F | 4 6 | Yes | 075::H? |
| 18 | ♂ K. | M | 0 2 | N | Not done |

01::H– means *E coli* O-antigen 1 and immotile, no H-antigen. The empty space between the two colons is for the K-antigen. The K-antigens have not been examined in this study

[b] This strain was only examined in Dr Lincoln's laboratory

H? means motile but not agglutinated with the established 49 H test sera.

TABLE 6 *Sex, age, previous history and serological E. coli type in 5 patients belonging to group 2 (higher titres against homologous antigen than against PA).*

| Case no. | Patient | Sex | Age (year/month) | Earlier urinary tract inf | *E. coli* type |
|---|---|---|---|---|---|
| 19 | ♂ A. | M | 0 y 2 mo. | N | 02r:H4 |
| 20 | S. L. | M | 2 2 | Yes | 026, (0133)..H |
| 21 | ♂ P | M | 0 1 | N | 076..H |
| 22 | U J | F | 3 5 | Yes | 0115..H– |
| 23 | B R. | M | 0 3 | No | Klebsiella |

026,(0133) means strong reaction in the former and weak reaction in the latter 0-serum.

means of the homologous antigens and the PA (see below) The results of the typings of the infecting *E coli* strains given in Tables 5–7 were unknown when the patients were grouped. There was full agreement between the results from the two bacteriological laboratories except in one patient, Case no. 99 which was found to be rough by Dr Lincoln and to be an 02..H– by Drs. Ørskov

In group 1 Table 5 and Fig. 3 comprised of eighteen patients, the difference

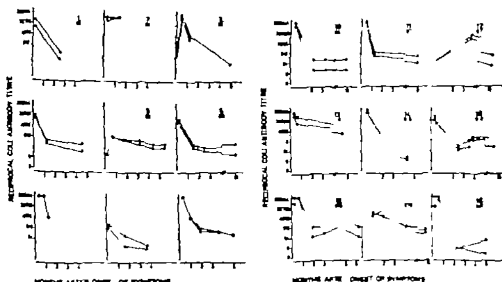



Fig 3 and 3b The course of the coli antibody titre in patients belonging to group 1 The 0 types of the employed homologous antigens are given at the curves. Full circles, titres obtained with the homologous antigen; open circles, titres obtained with the PA. Arrows indicate time of recurrences in Cases 15 and 16.

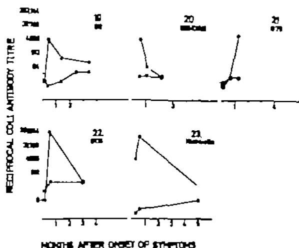


Fig. 4. The course of the coli antibody titre in patients belonging to group 2. The 0 types of the employed homologous antigens are given at the curves. Full circles, titres obtained with the homologous antigen; open circles, titres obtained with the PA.

between the titres found were three steps or less in all serum samples tested with the exception of the last sample in Case no. 18 where the difference was four steps. The concordance between the titres in this group is easily understood when the results are compared with the results of the typings of the homologous antigens, which all came out to belong to one or another of the 0-types, included in the PA.

In the other eleven patients the differences between the titres in the acute phases of the infections were greater than could be expected to be due to random errors [4]. They fall naturally into two groups, group 2 (five patients) with the higher titre obtained by means of the homologous antigens (Table 6 and Fig. 4), and group 3 with the higher titre against the PA (Table 7 and Fig. 5).

In group 2, four of the five strains isolated from the patients were found to be

TABLE 7. *Sex, age, previous history and serological* E. coli *type in 6 patients belonging to group 3 (higher titres against PA than against the homologous antigen)*

| Case no. | Patient | Sex | Age (year/month) | Earlier urinary tract inf. | *E. coli* type |
|---|---|---|---|---|---|
| 24 | U. K. | F | 10 y 1 mo. | Yes | 02nH4 |
| 25 | A. J. | F | 0 9 | N | 02nH |
| 26 | L. K. | F | 8 | Yes | 03nH |
| 27 | B. L. | M | 9 6 | N | 04,018, 023,0106nH |
| 28 | A. G. | F | 5 8 | Yes | (061), (055)nH4 |
| 29 | H. L. | F | 0 4 | N | rough/ 02nH |

This strain was rough according to Dr. Lincoln and 02nH– according to Drs. Orskov

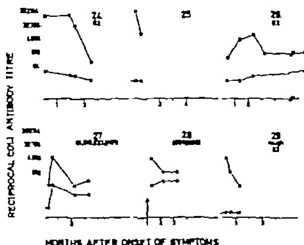



Fig. 4. The course of the coli antibody titre in patient belonging to group 3. The 0 types of the employed homologous antigens are given at the curves. Full circles, titres obtained with the homologous antigen; open circles, titres obtained with the PA. Arrow indicates time of recurrence in Case 28.

different from any of the types of the PA. The fifth was an 02.

In group 3 two of the homologous strains were different from the PA types (Cases no 28 and 29), while three belonged to serogroup 02. One strain (Case no 27) had partial relationship to two of the PA types.

## Discussion

The possibility of demonstrating specifi antibodies to one of two antigens, simultaneously adsorbed to red cells was first observed by Neter *et al.* [16]. They found that antisera against 055 or 0111 were agglutinated to the same titre whether the red cells were sensitized with one or both of the corresponding antigens. They concluded that each red cell had adsorbed both antigens, since incubation with either antiserum led to complete agglutination, which was not accomplished when the antiserum was added to a portion of red blood cells where only half of the cells were sensitized, the other half unsensitized.

In 1954 Landy [15] performed a series

Fig. 5. The course of the coli antibody titre during 5 years in patient A. J (See Discussion). The first arrow indicates the beck p at which her asymptomatic bacteruria was detected and the second arrow the time of her following asymptomatic relapse.

of similar experiments, using six polysaccharide antigens prepared from different bacterial species. He found the same serological reactivity regardless of whether the antigens were adsorbed individually si multaneously or consecutively Further he found that it was not possible to satu rate the cell receptors with a high con centration of a single antigen and therefore concluded, that the possibility of blocking out other antigens by a high concentration of any single one was unlikely

The increased interest in recent years in typing of *E coli*, isolated from different *sources in normal and pathological con* ditions has revealed that certain serotypes are more often encountered in disease than others. Thus, only a few types have been found to cause gastroenteric infections [23]. Ujváry [26] found that 63.5 % of *E coli* strains, causing different paren teral infections, belonged to the 0-groups 1–25 and Grönroos *et al.* [10] found a similar distribution in 76 women with urinary tract infections. Rantz [20] found that the 0-groups ² 4 6 and 75 constituted 49.3 % of the bacteria in 156 patients with urinary tract infections. Turck *et al.* [24] found 0-groups 1 4, 6 7 and 75 in 61 1 % of groupable strains in urinary tract in fections, and recently Vosti *et al.* [27] reported, that as many as 82 % of groupable strains in 78 women with recurrent urinary tract infections belonged to the 0-groups 1 4, 6 7 16/62 25 50 and 75 Preliminary results from a similar investi gation in children with urinary tract in fections in progress in our group [17] are in agreement with these results. Thus it seems obvious that, although most coli types may be encountered in urinary tract infections, there is a prevalence for some few types. Whether this depends upon a particular nephropathogenicity of those groups or merely upon the more common occurrence in the environment is still a matter of dispute.

The selection of 0-types included in the PA in this study is mainly based on the results of our own investigation, which seems to be in agreement with most others, although it appears probable that some geographic or age-dependent differences may exist.

It may be possible that more than eight antigens can be adsorbed to the same por *tion of blood cells. This, however has not* been investigated in this study because the different studies concerning the frequency of *E coli* types in urinary tract infections all show that the relatively few infections, not caused by the common types, are caused by a very wide distribution of types in low frequencies.

The results of the experiments per formed on rabbit *E coli* antisera in the present study are in agreement with those of Neter *et al* [16] and Landy [15]. It was possible to demonstrate that the employed eight different *E coli* 0-antigens were adsorbed to sheep red blood cells by simultaneous sensitization with a mixture of the antigens and that the titres deter mined by means of this mixture compared well with the titres obtained with the antigens used individually Because of the difference in concentrations between an antigen, used for separate sensitization, and the same antigen used in the mixture (i.e. 8 1) the PA might be expected to give a lower titre than the separate antigens. However no such discrepancy was observed. This may be explained by the fact that, with both concentrations a

great excess of antigen is used for sensitisation [*].

The specificity of the PA for demonstrating antibodies to any single one of the antigens included is demonstrated by the low titres of the cross-reactions and by the almost total inhibition of the hemagglutination reaction found when the antisera were preincubated with their corresponding antigens.

The possible influence of antibodies to Kunin's common enterobacterial antigen [13, 14] was investigated by testing the antisera with a crude preparation of this antigen, prepared from *E. coli* 014. The titres against this antigen were very low. This is in accordance with the findings of Gorzynski *et al.* [9], who found that antibodies to this antigen could only be elicited in rabbits with the help of adjuvants.

In the clinical part of this study elevated antibody titres to PA were found in twenty-four out of the twenty-nine patients. In the patients belonging to group 1 this might be expected as the serological typings of the homologous strains revealed that they all belonged to one or another of the 0-types included in the PA. The concordance between the results obtained with the PA and the patients' homologous urinary antigens was good.

In the five patients in group * the titres against the homologous antigens were higher than against the PA. With reservation for the patient infected with an 02 strain, this result is in full agreement with the results of the serological typing.

The reverse discrepancy between the titres in group 3 is very interesting. Although these patients were found to be infected with strains not leading to raised titres they had elevated titres against the PA.

Case no. 24 is a patient with enuresis and repeated attacks of febrile urinary tract infections. The actual infection was an afebrile one with frequent and burning micturitions. Urinary analysis revealed heavy leucocyturia and 50 000 *E. coli* per ml of urine on culture.

Case no. 26 had at the actual infection asymptomatic bacteriuria after several earlier incidences of acute pyelonephritis. She is suspected of having a chronic pyelonephritis because of a constant reduction of the renal concentrating capacity and the roentgenologic findings of papillary necrosis in the right kidney and heavy vesico-ureteric reflux on the same side.

Case no. *8 had at the actual infection an overt pyelonephritis, but only 5 000 *E. coli* (051),(055) .H4 were found in the urinary culture. However, she had one month earlier been treated for an acute pyelonephritis caused by a strain belonging to the type 07 .H–. This strain was not available for antigen preparation.

In these three cases it seems reasonable to believe that the titres against the PA are the results of earlier challenges with strains, belonging to one or another of the PA types. The types of the infecting strains at the earlier infections in Cases no. 24 and 26 are unfortunately not known.

In Case no. 25 no earlier infection could be traced from the case history.

In Case no. *7 the curves obtained by titrations with the homologous antigen and with the PA are parallel, although at different levels. This may reflect the partial relationship between the homologous antigen and two of the types included in the PA.

In Case no. 29 the titres found with the infecting strain were on all occasions zero

of similar experiments, using six polysaccharide antigens, prepared from different bacterial species. He found the same serological reactivity regardless of whether the antigens were adsorbed individually simultaneously or consecutively. Further he found that it was not possible to saturate the cell receptors with a high concentration of a single antigen and therefore concluded, that the possibility of blocking out other antigens by a high concentration of any single one was unlikely.

The increased interest in recent years in typing of *E. coli* isolated from different sources in normal and pathological conditions has revealed that certain serotypes are more often encountered in disease than others. Thus, only a few types have been found to cause gastroenteric infections [23]. Ujváry [26] found that 63.5 % of *E. coli* strains, causing different parenteral infections, belonged to the 0-groups 1–25 and Grönroos *et al.* [10] found a similar distribution in 76 women with urinary tract infections. Rantz [20] found that the 0-groups 2, 4, 6 and 75 constituted 49.3 % of the bacteria in 156 patients with urinary tract infections. Turck *et al.* [24] found 0-groups 1, 4, 6, 7 and 75 in 61.1 % of groupable strains in urinary tract infections and recently Vosti *et al.* [27] reported, that as many as 82 % of groupable strains in 78 women with recurrent urinary tract infections belonged to the 0-groups 1, 4, 6, 7, 16/6..., 25, 50 and 75. Preliminary results from a similar investigation in children with urinary tract infections in progress in our group [17] are in agreement with these results. Thus it seems obvious that although most coli types may be encountered in urinary tract infections, there is a prevalence for some few types. Whether this depends upon a particular nephropathogenicity of these groups or merely upon the more common occurrence in the environment is still a matter of dispute.

The selection of 0-types included in the PA in this study is mainly based on the results of our own investigation, which seems to be in agreement with most others, although it appears probable that some geographic or age-dependent differences may exist.

It may be possible that more than eight antigens can be adsorbed to the same portion of blood cells. This, however has not been investigated in this study because the different studies concerning the frequency of *E. coli* types in urinary tract infections all show that the relatively few infections, not caused by the common types, are caused by a very wide distribution of types in low frequencies.

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great excess of antigen is used for sensitization [*].

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In the five patients in group 2, the titres against the homologous antigens were higher than against the PA. With reservation for the patient infected with an O* strain, this result is in full agreement with the results of th serological typing.

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Case no. 28 had at the actual infection an overt pyelonephritis, but only 5 000 *E. coli* (O51),(O55):H4 were found in the urinary culture. However she had one month earlier been treated for an acute pyelonephritis caused by a strain belonging to the type O7 H–. This strain was not available for antigen preparation.

In these three cases it seems reasonable to believe that the titres against the PA are the results of earlier challenges with strains, belonging to one or another of the PA types. The types of the infecting strains at the earlier infections in Cases no. 24 and 26 are unfortunately not known.

In Case no. 25 no earlier infection could be traced from the case history.

In Case no. 27 the curves obtained by titrations with the homologou antigen and with the PA are parallel, although at different levels. This may reflect the partial relationship between the homologous antigen and two of the types included in the PA.

In Case no. 20 the titres found with the infecting strai were on all occasions zero,

while the titres against the PA showed a definite elevation at the beginning of the disease The homologous strain was found to be rough by one laboratory (Lincoln) and to belong to group 02..H– by the other (Ørskov) *The infection may thus* have been caused by a mixture of rough and smooth strains and the homologous antigen may by chance have been prepared mainly from rough colonies. Thus, the antibodies (to 02?) could not be detected by means of the homologous antigen preparation, while they were found with the use of the PA.

The validity of the assumption that earlier *E coli*-infections may be the cause of elevated titres to the PA may be illustrated by the course of the coli antibody titres in a patient, not otherwise included in this study (Fig 6) It is a girl with a chronic pyelonephritis followed in different periods during five years She was described in an earlier study [4] as Case no 17 group 1 Originally she was infected with a strain, identified as 01 H– leading to typical findings for an acute pyelonephritis Eleven months after the first infection she was suspected to have a relapse Since then she has been feeling well. However on a routine check up in January 1966, four years after the original infection she was found to have >10 mill. *E coli* 02 H1 and pyuria. After treatment the bacteriuria disappeared but 2 months later she had a new asymptomatic infection, now with *E coli* 083 032..H?

At all check ups her titre against the original 01 infection has been elevated. The titre against the PA has been followed since the check up in January 1966 and has been practically identical with the 01 titre The titre against the 083 032-strain, however has been diverging from the other titres. Thus it appears possible, that a previous infection with a strain belonging to the PA types, may lead to a continuous elevation of the PA-antibody titre Whether this is due to a chronic infection in the kidneys, to persistence of bacterial variants [11] to the presence of antigen in antibody producing tissue [7 ?1] or to continuous endotoxin stimulation [25] is not yet clear

The present study has revealed that it is possible to replace the use of the homologous strains for titration of the antibodies against *E coli* in urinary tract infections with a standard set-up of the most commonly encountered 0-types. The use of such a standard, adjusted to the local differences in frequencies of infecting types, seems to be a practical advantage to the use of strains, isolated from each individual patient. Further it seems to be *of particular value in* the evaluation of patients with suspected urinary tract infections but with inconclusive urinary findings, and in patients with suspected chronic pyelonephritis without bacteriuria. An extensive survey of patients who have earlier had acute urinary tract infections is now in progress at this hospital and may further elucidate these problems [5].

## Summary

Hemagglutination experiments were performed in rabbit *E coli* antisera against *E coli* 0 types 1 2, 4, 6 7 8, 18 and 75. The results of titrations with sheep red blood cells, modified with all the corresponding eight antigens, were equal to the results found with cells modified only with the corresponding antigen. The specificity

of the reaction between each of the antisera and the polyvalent antigen (PA) were investigated by means of hemagglutination inhibition experiments which showed that almost no hemagglutination was obtained when the antisera were preincubated with their corresponding antigen.

Serum specimens were collected from 29 children with urinary tract infections and titrated with the PA as well as with antigen prepared from the patients' own urinary bacteria. Similar titres with the two antigen preparations were found in 18 patients. Serotyping of the urinary flora revealed, that all strains from these patients belonged to one or another of the O-groups, included in the PA. In five patients higher titres were found with the homologous antigen than with the PA and four of the homologous strains were different from the PA O-groups. Six patients had elevated titres against the PA, but normal titres against their homologous antigens. This is believed to be due to earlier infections with strains belonging to the PA.

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# Bronchial Asthma and Recurrent Pneumonia in Children

## *Clinical Evaluation of 14 Children*

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Bronchial asthma is sometimes complicated by more or less extensive pulmonary atelectasis [1 4 16, 17 35] and in patients with an asthmatic attack of 24 hours or more an intercurrent pneumonia is sometimes found [?7]. Atelectasis may predispose to organic bronchial changes. The connection between atelectasis and bronchiectasis has been the subject of many investigations [3 10 1.. 18].

In childhood, pneumonia seems to be the chief predisposing factor to bronchiectasis and more common than bronchial asthma *per se* [9 1.., 14]. It is thus clear that obstinate pneumonia can give rise to later chest disease. Furthermore, even if clinical and roentgenological examination show nothing remarkable, function studies may reveal impairment of pulmonary function long time after the acute stage of a pneumonia [5 7 23, 32].

It is well known that pneumonias may be secondary to organic defects of the bronchi or to diseases such as mucoviscidosis and a defective immune response, in which conditions asthmatic symptoms are sometimes seen. Such pneumonia is prone to relapse. As mentioned above, bronchial asthma might also predispose to pneumonia. It was therefore thought legitimate to study the clinical relation between bronchial asthma and recurrent pneumonia in children.

Children with bronchial asthma and recurrent pneumonia were therefore investigated with regard to clinical symptoms and signs, etiology of the disease, prognosis and lung function.[1]

### Material

Bronchial asthma is, as usual, to be understood as recurrent episodes of bronchial obstruction producing the well known symptoms of dyspnoea with expiratory wheezing; recurrent pneumonia, as 2 or more episodes of respiratory distress with roentgenologically verified pulmonary parenchymal process.

In January 1966, 125 asthmatic children, 6 to 14 years of age, were on the register of the department of pediatrics, University Hospital, Lund. Of these 125 children, 3 boys and 11 girls fulfilled the criteria of recurrent pneumonia. They were reinvestigated when during remission in 1966 and the first half of 1967. None of the 14 children had received steroid treatment.

### Methods

*Chest X-ray.* Lateral and frontal views of all 14 children were carefully re-examined in

[1] Results of lung function studies in these children will be reported elsewhere.

TABLE 1 *Some characteristics of the basic material and the children with bronchial asthma and recurrent pneumonia.*

| Material | Males % | Atopic heredity % | Eczema % | Verified allergy % | Age at onset of asthma (years) 0–3 % | 3½–7 % | >7 % |
|---|---|---|---|---|---|---|---|
| Basic (125 children) | 50 | 74 | 51 | 57 | 52 | 41 | 7 |
| 14 children with asthma and recurrent pneumonia | 21 (3/14) | 79 (11/14) | 36 (5/14) | 50 (7/14) | 43 (6/14) | 50 (7/14) | 7 (1/14) |

Atopic heredity—heredity for eczema (Prurigo Besnier) and for seasonal rhinitis.
[b] Eczema infantum—atopic eczema—eczema Prurigo Besnier

collaboration with an experienced roentgenologist. The relative heart volume was estimated according to Jonsell [22].

*Allergological tests.* In the course of their disease all 14 children had been subjected to intracutaneous tests with house-dust, pollen, animal danders and moulds. The highest concentration of the allergens used was 1 1000 (w/v). Intracutaneous tests with foods were not performed routinely. A positive intracutaneous test or a history of suspected allergy despite a negative intracutaneous test was considered sufficient to justify inhalation tests, which were continued until an asthmatic reaction developed or t a maximum volume of 1 ml allergen with a concentration of 1 10.

*Sweat electrolytes.* Sweat was obtained by the pilocarpine cataphoretic method of Gibson & Cooke [13]. Sodium was estimated with the flame photometric method and chloride with the method of Schales & Schales [30].

*Serum proteins.* Paper electrophoresis was used for quantitative determination of albumin, $\alpha 1$, $\alpha 2$, $\beta$ and $\gamma$-globulins.

*Fecal chymotrypsin* was estimated according to Haverback *et al.* [19].

## Results

Of the 125 asthmatic children, 14 (11.2 %) had recurrent pneumonia. No appreciable differences were found between the series as a whole and the asthma-pneumonia group regarding common characteristics such as allergic heredity atopic eczema verified allergy and age at the first attack of asthma (Table 1). The sex distribution of the asthma-pneumonia group (21 % males) was, however unusual and differed from that of the total material.

Characteristics of the allergy and pneumonia of the 14 patients are given in Tables 2 and 3. The severity of the asthma is classified according to Engström & Kraepelien [11] group 1 mild asthma or less than 5 attacks per year group 2: moderate-severe asthma or 5–10 attacks per year and group 3 severe asthma or more than 10 attacks per year. According to this classification 8 subjects had severe asthma, 5 had moderate-severe asthma and only 1 mild asthma during their worst year. During the year of the present review 10 patients had mild asthma, 1 moderate-severe and only 3 severe asthma. Verified allergy was established in 7 children, 5 of whom had received specific hyposensitization.

In 9 cases asthmatic attacks preceded or coincided with the first occurrence of

I am greatly indebted t Wigher Mortensson, M.D. Department II of Radiology University Hospital, Lund.

TABLE _ *Allergological characteristics of 14 children with bronchial asthma and recurrent pneumonia.*

| Case | Sex | Age at onset of asthma | Age at follow up | Atopic heredity* | Other topic diseases | Verified allergy | Specific desensitization | Severity of asthma[b] Worst ear | Severity of asthma[b] Follow-up year |
|---|---|---|---|---|---|---|---|---|---|
| 1. K. P | F | 4/12 | 7 | No | No | Yes | Yes | 2 | 1 |
| 2. B. A | M | 2 | 10 | + + | No | [illegible] | No | | 1 |
| 3. G. B. | F | 5⁶/12 | 10 | + + | No | Yes | Yes | 1 | 1 |
| 4. P B. | F | 3⁴/12 | 10 | + | No | Yes | No | | 1 |
| 5. G L. | M | 1 | 7 | + | Eczema | No | No | 3 | 1 |
| 6. G. J | F | 1 | 11 | + | Eczema | Yes | Yes | 3 | 3 |
| 7 M. P | F | 6⁶/12 | 9 | + + | Eczema | No | No | 3 | 1 |
| 8. M. N | F | 2⁴/12 | 10 | + | Eczema | Yes | Yes | 3 | 1 |
| 9 H. B. | F | 6 | 13 | + | No | No | No | 2 | 1 |
| 10 J P | M | 4/12 | 8 | + + | No | Yes | Yes | 3 | 3 |
| 11. L. L. | F | 1/12 | 9 | No | No | No | No | 2 | 1 |
| 12. A. A. | F | 4 | 11 | + + | No | No | No | 3 | 2 |
| 13. B. W | F | /12 | 6 | + | Eczema | Yes | No | 3 | 1 |
| 14. C. A. | F | 5⁴/12 | 7 | No | No | No | No | 3 | 3 |

\+ = Heredity in the family of the mother or the father + + = Heredity in the family of both the mother and the father

Severity of asthma according to Engström & Kraepelien [11].
1 less than 5 attacks per year; 2, 5–10 attacks per year; 3, more than 10 ttacks per year

pneumonia In 5 cases the asthmatic symptoms began after the first occurrence of pneumonia. Three of these patients had, however previously had other atopic manifestations and/or had heredity In the remaining cases there was no known heredity for atopic disease and neither verified allergy nor other atopic diseases could be demonstrated (nos. 11 and 14)

The 14 subjects had all together 64 attacks of radiographically verified pneumonia and 6 clinically diagnosed attacks i.e. on the average 5 0 per subject. Most of the attacks were combined with severe bronchobstructive symptoms and in 2 patients (nos. and 12) one ttack was complicated by massive collapse of one of the lungs.

The ESR was increased in 70 %, fever was recorded in 87 %, leucocytosis in 47 % and pronounced relative eosinophilia (> 10 %) during *0 % of the attacks of pneumonia (Table 3)

The distribution of the lobes involved by the pneumonia is given in Table 3 and Fig 1 The overrepresent tion of the middle lobe is striking In 18 attacks in 9 patient both the middle lobe and the lingular area were affected. In 20 attacks (in 11 patients) both lungs were involved simultaneously Only in 1 patient were the pulmonary changes never bilateral. In most patients pulmonary areas affected differed from one ttack to another In 4 children the roentgenological changes in the middle lobe persisted for months or more In 6 patients new parenchymal processes without clinical symptoms ap-

* Such determinations were unfortunately not alw ys performed in the acute stage

TABLE 3 *Characteristics of the pneumonias in 14 children with bronchial asthma and recurrent pneumonia*

| Case | Age at first pneumonia | Number of attacks of pneumonia | | Affected lung | | Chron. pulm. changes (> 2 months) | Result of bronchography | Number of occurrences with symptoms of | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | x-ray | No x ray | Right | Left | | | Increase of ESR[b] | Fever > 38 C | Leuco-cytosis > 12,000 | Rel. eosinophilia 5–9% | Rel. eosinophilia > 10% |
| 1 | 4 / | 2 | 0 | 2 | 1 | N | Not performed | 1 | 1 | 0 | 1 | 1 |
| 2 | 1 /12 | 2 | 1 | 2 | 0 | N | Not performed | 1 | 1 | 0 | 1 | 0 |
| 3 | $5^{9}/$ | 3 | 0 | 3 | 2 | No | Not performed | 0 | 3 | 1 | 1 | 1 |
| 4 | $3^{6}/_{12}$ | 3 | 0 | 3 | 2 | N | Not performed | 3 | 0 | 0 | 2 | 1 |
| 5 | 4 / | 3 | 1 | 3 | 1 | Middle lobe | Atelectasis middle lobe | 3 | | 0 | 2 | 0 |
| 6 | 1 | 3 | 1 | 3 | 2 | Middle lobe | Atelectasis middle lobe | 3 | 4 | 2 | 0 | 2 |
| 7 | $6^{9}/_{12}$ | 4 | 0 | 4 | 1 | N | Normal | 3 | 3 | 2 | 3 | 0 |
| 8 | 3 /12 | 4 | 1 | 4 | 3 | Middle lobe | Bronchiect. middle lobe | 1 | 4 | 3 | 0 | 0 |
| 9 | 6 / | 4 | 1 | 3 | 3 | N | Normal | 1 | 4 | | 0 | 0 |
| 10 | /12 | 5 | 0 | 5 | 3 | N | Normal | 2 | 3 | 2 | 3 | 1 |
| 11 | /12 | 5 | 1 | 5 | 4 | Middle lobe | Not performed | 2 | 3 | 2 | 1 | 2 |
| 12 | $4^{10}/_{12}$ | 6 | 0 | 5 | 2 | N | Normal | 6 | 6 | 3 | 0 | 0 |
| 13 | 1 /12 | 8 | 0 | 8 | 6 | No | Not performed | 8 | 8 | 3 | 0 | 1 |
| 14 | 4 /12 | 11 | 0 | 11 | 3 | N | Segment. atel. upper and lower lobes | 0 | 11 | 9 | 1 | 0 |

First pneumonia preceded onset of asthma.
[b] Increase of ESR > 15 mm.

peared during the healing stage of the attack of pneumonia for which the patient had been admitted.

Bronchography was done in 8 cases. In one child with chronic middle lobe engagement (case 8) the bronchus of that lobe was found to be narrowed (Fig 2) Re-examination 1½ years later showed that the bronchus was not quite as narrow as before. It is not known whether the narrowing was due to chronic peribronchial inflammation, enlarged lymph nodes or congenital anomaly Bronchography also revealed a varying degree of reduction of the parenchyma of the middle lobe in 3 children (cases 5, 6 8) and atelectasis of the posterior segment of the right upper lobe and the apical segment of the right lower lobe in 1 child (case 14). None of the patients were found to have bronchiectasis.

In only 1 case was body height and weight below the lower 2-sigma limit (no 6) Investigation of this child for endocrine disturbance revealed nothing remarkable She had for a long time chronic asthma of increasing severity and the growth disturbance was ascribed to her asthma

Cystic fibrosis of the pancreas, defective immune response bronchial anomalies

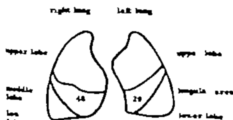



Fig 1 Number of attacks of pneumonia with involvement of the various lung lobes in 14 children with bronchial asthma and recurrent pneumonia

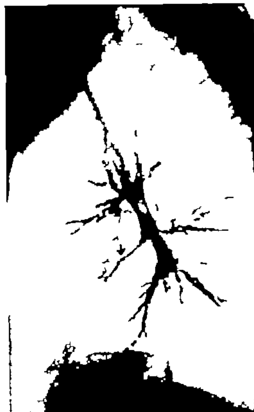


Fig. 2. Bronchogram of case 8. The narrowed bronchus of the middle lobe is indicated with an arrow

and bronchial foreign body were often suspected but could never be verified (Table 4) Heart disease and tuberculosis could also be excluded

One child had since early childhood had short periods of 2 loose stools a day (case 2) But the results of investigations for malabsorption disturbance were negative. He had a deficiency of serum Ig A, which was considered unrelated to his disease

## Discussion

In the present investigation no distinction was made between atelectasis and pneumonia owing to the difficulty in clini

TABLE 4. *Fecal chymotrypsin, sweat electrolytes, serum $\alpha_1$ and $\gamma$-globulins and blood eosinophils at review*

| Case | Fecal chymotrypsin (μg/g) | Sweat electrolytes[b] (mEq/l) | | Serum proteins[c] (g/100 ml) | | Blood eosinophils[d] per mm³ | |
|---|---|---|---|---|---|---|---|
| | | N | Cl | α1 | γ | | |
| 1 | 45 | 12.3 | 16.5 | 0.24 | 0.91 | 669, | 819 |
| 2 | 293 | 16.7 | 8.2 | 0.20 | 0.64 | 250, | 369 |
| 3 | 345 | 12.0 | 15.7 | 0.26 | 1.14 | 306, | 444 |
| 4 | 310 | 77.9 | 36.7 | 0.41 | 1.16 | 894 | 513 |
| 5 | 267 | 13.8 | 19.5 | 0.22 | 0.63 | 494 | 900 |
| 6 | 650 | 16.8 | 6.2 | 0.25 | 1.28 | 476 | — |
| 7 | 306 | 35.1 | 35.6 | 0.20 | 0.76 | 119, | 1176 |
| 8 | 243 | 56.0 | — | 0.24 | 0.75 | 344 | 706 |
| 9 | 468 | 58.5 | 26.0 | 0.21 | 1.07 | 788, | 1044 |
| 10 | 278 | 50.0 | 37.5 | 0.21 | 0.88 | 1263, | 784 |
| 11 | 276 | 65.0 | 19.0 | 0.25 | 0.78 | 156, | 181 |
| 12 | 585 | 39.0 | 50.0 | 0.28 | 0.63 | 251 | 460 |
| 13 | 580 | 61.7 | 24.1 | 0.39 | 1.01 | 538, | 328 |
| 14 | 636 | 39.8 | 22.3 | 0.27 | 0.60 | 380, | 786 |

Lower normal limit of fecal chymotrypsin is 74 μg/g feces.
Upper normal limit of sweat sodium is 80 mEq/l and of sweat chloride 60 mEq/l.
Serum $\alpha_1$ antitrypsin was determined at the Clin. Chem. Dpt., Malmö (Carl-Bertil Laurell, M.D.) and proved normal. Quantitative determinations of IgA, IgG and IgM were performed at the Bact. Dpt., University Hospital, Lund, by A. B. Laurell, M.D. and V. Oxelius, M.D. Case 2 had an IgA deficiency.
Blood eosinophils were estimated on 2 occasions during remission.

cal and roentgenological differentiation between these conditions in patients with asthmatic symptoms. Acute pneumonia may be combined with more or less atelectatic changes; furthermore the clinical and laboratory symptoms of acute pneumonia and acute atelectasis are often the same [6, 8, 18, 34]. Experimental atelectasis in dogs is accompanied by fever, tachycardia and tachypnoe; cultivation of material from the atelectatic area will reveal secondary infection within 1_ hours. Prophylaxis with antibiotics prevents such infection [21, 24].

In the present material most of the episodes of respiratory distress with parenchymal processes of the lung were preceded by catarrhal symptoms, combined with fever, increased ESR and leucocytosis. It is true that catarrhal symptoms per se may be of allergic origin and that fever may be a manifestation of the heavy respiratory work. But in view of the clinical picture as a whole these episodes were interpreted as being of infectious nature. As mentioned above it is not possible to decide whether a pulmonary infection is a primary cause of the symptoms or a bronchobstructive stage leads to atelectasis with secondary infection. Probably both mechanisms were involved. About 11 % of asthmatic children in the present study had recurrent pneumonia. This is, no doubt, a minimum value, because chest x-ray was not performed in every case of bronchobstructive distress admitted to the hospital. It may be mentioned that the frequencies of 1.3 to 7.4 % have been given

for pulmonary atelectasis in children with asthma [25 26 28 35]. In 2 of these studies unspecific parenchymal processes were reported in further 4 and 13 % [26 35] and in one of them recurrences were reported in about 7 % [25]. It is of great interest that among these patients with recurrences a strong female preponderance was observed, which is in agreement with the present study

The tendency to recurrences was strong of 14 patients had 5 or more attacks of pneumonia. These frequent relapses and the appearance of new parenchymal processes without clinical symptoms in the healing stage of the primary pneumonia are often suggestive of cystic fibrosis of the pancreas. It may be mentioned however that while these 14 patients were treated in the department only 9 cases of cystic fibrosis of the pancreas were diagnosed. This clearly indicates that bronchial asthma is a more common cause of recurrent pneumonia than mucoviscoidosis. It should, however be mentioned that cystic fibrosis of the pancreas is less common in Sweden than in certain other countries such as USA [31].

The frequency of common allergic characteristics was the same in the 14 children with asthma and recurrent pneumonia as in the entire material (1_5 asthmatic children) These figures re also in general agreement with corresponding figures in other reports [11 29] During optimal stage most of the children had eosinophilia which further supports the diagnosis of asthma.

Pneumonia, pertussis and pulmonary collapse are the commonest conditions predisposing to bronchiectasis [9 1 14]. It is difficult to estimate the incidence of bronchiectasis in chronic asthma of children but it is presumably low [2, 26 28 37] Asthma combined with recurrent pneumonia probably favours the development of bronchiectasis more than either of these conditions alone Though the present material is too small to allow any conclusion regarding the relation between these 2 conditions, it is astonishing that none of the children had clinical or roentgenological signs of bronchiectasis. Residual atelectasis was, however noted in 4 cases, which may indicate a risk of later bronchiectasis. In only one case was a bronchial lesion diagnosed (case 8)

As in most published series atelectasis showed a predilection for the right lung especially the right middle lobe [12 15 18 25 26 34]. The lingular part of the upper lobe was the most frequently affected part of the left lung The lingular part being the left homologue to the middle lobe it is also frequently affected by ordinary pneumonia and atelectasis [20, 36].

No explanation can be offered for the frequent relapses of pneumonia It is possible that residual organic changes after an episode of pneumonia may add to the general bronchial weakness of the asthmatic child.

In most of the children the frequency of asthmatic attacks decreased with increasing age, possibly owing to the gradual increase in width of the bronchi The latter prognosis is, however uncertain owing to the risk of recurrence and of later chest disease such as bronchiectasis.

## Summary

Of 1_5 children with bronchial asthma 14 had recurrent pneumonia (11_ %). 7 had 5 or more relapses. An overrepresenta-

tation for the right lung and its middle lobe was found. Of the left lung the lingular part was most frequently affected. Simultaneous involvement of the middle lobe and the lingula was found on 18 occasions. No underlying diseases, except bronchial asthma were found. Of other diseases, cystic fibrosis of the pancreas and abnormal immune response to infection could be excluded. Positive support for the diagnosis of bronchial asthma was found, viz. characteristics such as atopic heredity other manifestations of atopic disease verified allergies and age at onset of the bronchobstructive attacks were equally common in the basic material as in the asthma pneumonia group.

Bronchographic investigations of 8 subjects revealed a bronchial abnormality in only 1 case The results are discussed in relation to earlier investigations and it is stressed that in children bronchial asthma is often combined with recurrent pneumonia. The early prognosis of asthma in this group of patients seems to be good but the late prognosis is uncertain.

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# Glucose Cholesterol and Creatinine in the Foetal Tracheobronchial Fluid of the Guinea Pig and the Sheep

by TERHO O A. PAAVILAINEN LEO HIRVONEN and TUOMAS PELTONEN

The concentration of different substances in the amniotic fluid is a result of a dynamic equilibrium There is interaction not only between the amniotic cavity and the mother animal but also between this space and the foetus [4 14 17] It is well known that the foetus may swallow amniotic fluid and excrete into it especially under asphyctic conditions [11] The transfer of substances from the foetus into the amniotic fluid or in the opposite direction has been less well studied. The composition of the liquid contained in the foetal respiratory tract is not the same as that of the amniotic fluid [1] It has been suggested that transudation of liquid occurs through the foetal alveoli from the blood [16 18]. Large quantities of liquid have been collected by tracheal cannula from lamb foetuses with intact umbilical circulation. Adams *et al* [ ] stated that the composition of this fluid is in most respects similar to that of the foetal blood and concluded that the foetal tracheal fluid originates in the lower airways

The present study was undertaken to obtain more information about the composition of tracheobronchial fluid. A summary of the results has already been published [7 1* 13]. The concentration of sugar cholesterol and creatinine were determined in the tracheobronchial and amniotic fluid and foetal and maternal blood of the guinea pig and the sheep

## Methods

Seven pregnant sheep and 11 guinea pigs were used as the experimental animals. Most animals were near term. The sheep had altogether 15 foetuses (weight 1 6 to 4.0 kg, average 2.3 kg) and the guinea pigs 42 foetuses (weight 40 to 115 g) Caesarean section was performed on the sheep under local anaesthesia with Xylocain (0 5 %). The guinea pigs were anaesthetized slightly with an intraperitoneal Nembutal injection (30 mg/kg of b.w ) and in addition to this local Xylocain anaesthesia was applied to the abdominal wall. Before removing a foetus from the uterus an amniotic fluid sample was taken. The foetus was then delivered. The heads of the lamb foetuses were covered with rubber condoms to prevent the initiation of respiration. In the guinea pig foetuses their own foetal membranes served this purpose. After incision of the skin on the neck, samples of the foetal tracheobronchial fluid were taken by punctures of the trachea. The cranial end of the trachea was occl ded to prevent a flow of the fluid from the mouth and nasal cavities. A sample of 5 t 10 ml was btained from the lambs and one of 0.1 to 0 6 ml from the guinea pig foetuses. Foetal blood samples were taken from the cord blood; if this

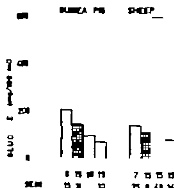


Fig. 1 Glucose concentrations in the various liquids. Striped columns: maternal blood. Lattiсed columns: foetal blood. Dotted columns: amniotic fluid. Clear columns: foetal tracheobronchial fluid.

was not successful as was the case in a few guinea pig foetuses, cardiac puncture was performed. Maternal blood was taken from a peripheral vein in the sheep and from the caudal caval vein in the guinea pigs.

The following analytical methods were used: glucose was determined according to Hultman as modified by Hyvärinen & Nikkila [9] and cholesterol as described by Pearson et al. and modified by Leppänen [10]. Creatinine was determined photometrically from a proteinfree sample after addition of alkaline picrat solution according to the Astra handbook [6].

## Results

The results of the *glucose* determinations are presented in Fig 1. The smallest glucose concentration in both species was observed in the tracheobronchial fluid. This concentration was lower than that of the maternal or foetal blood. In the amniotic fluid of the sheep the glucose concentration was more than four to five times as high as in the blood samples of the mother animal or of the foetus. In the guinea pig there was less glucose in the amniotic fluid than in the maternal or foetal blood. The concentration gradient between amniotic fluid and tracheal fluid which was so high in the sheep was only slight in the guinea pig. These gradients were however parallel.

The *cholesterol* concentrations of different fluids are seen in Fig 2. Here again considerable differences were obtained between the species. In the sheep there was more cholesterol in the maternal blood than in the foetal blood, in the guinea pig the position was reversed. In both species the cholesterol concentration of the tracheobronchial fluid was low compared with that of the blood samples. There were only 4 cases in the series of 15 sheep foetuses where the cholesterol concentration was more than virtually non-existent. In the sheep there was on an average 10 times as much cholesterol in the amniotic as in the tracheobronchial fluid, in the guinea pig the concentrations of these fluids were about the same. The age of the foetuses had no bearing on the concentration gradients of sugar and cholesterol.

The results of the *creatinine* determinations are reported in Fig 3. The results obtained for guinea pig of smaller foetal size differ from those of larger foetal size; the creatinine concentration of all the

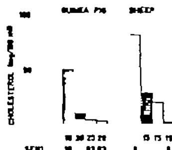


Fig. 2. Cholesterol concentrations in the various liquids. Designations as in Fig 1

liquids studied was higher for the former group. In addition to this there was more creatinine in the tracheobronchial fluid than in the serum of the smaller foetuses. In the larger foetuses the serum creatinine concentration was slightly higher than that of the tracheobronchial fluid. A comparison between creatinine contents of tracheobronchial fluid and maternal serum and these of amniotic fluid and foetal serum showed significant differences in the series of smaller foetuses. In the experiments with larger guinea pig foetuses there were hardly any differences. As for the sheep experiments the concentration gradients were in the same direction as those of the guinea pigs with smaller foetuses. The creatinine concentration in the amniotic fluid of the sheep was five times as high as that in the guinea pig series with smaller foetuses.



Fig. 3. Creatinine concentrations in the various liquids. Designations as in Fig. 1

## Discussion

Of the many constituents of the various fluids glucose was selected to represent easily transferable nutrients, cholesterol lipids and creatinine non-protein nitrogen substances. In the sheep the foetal blood sugar consists predominantly of fructose [3] this is not so in the guinea pig [5]. At this phase of the studies fructose determinations were not performed. A high fructose concentration may elevate the result of glucose determinations. The importance of this factor will be a subject of further studies. There are interesting species differences. Especially high sugar concentrations were obtained in the amniotic fluid of the sheep. In the guinea pig the glucose content of the amniotic fluid was lower than that of the maternal or foetal blood. In both species there was more glucose in the amniotic than in the foetal tracheobronchial fluid. The amniotic fluid is a pool of a variety of components each of which is in a well-ordered state of equilibrium with its surroundings and not an ultrafiltrate of serum [14]. For the formation of amniotic fluid the foetus is of significance: the exchange of substances between mother and amniotic fluid is direct or indirect (through the foetus). Tracheobronchial fluid reaches the amniotic cavity via the upper airways. The transfer of water and other substances from the foetus into the tracheobronchial fluid or in the opposite direction is possible through the pulmonary capillaries or through the walls of the lower airways.

According to Plentl the water exchange between the maternal organism and the products of gestation can be presented by a kinetic model compared of three major compartments, all of which exchange with each other. These compartments are the amniotic fluid, the maternal and the foetal body [14, 15]. The tracheobronchial fluid forms an intermediate stage in the transfer between amniotic fluid and foetus via the respiratory tract. This is not the

only way in which transfer occurs foetal skin, gastrointestinal tract and urinary organs must also be taken into consideration.

Adams *et al* [1] postulated that the foetal tracheal fluid originates in the lower foetal respiratory tract and suggest that the foetal tracheal fluid may be the result of ultrafiltration with selective reabsorption or ultrafiltration with selective secretion by the foetal lung

Creatinine was the only substance which occurred in a higher concentration in the foetal tracheobronchial fluid than in the foetal blood. This was true of the lamb and smaller guinea pig foetuses. Different creatinine concentrations in different phases of foetal development were interesting but at this stage of the study it is impossible to provide any explanation of this observation.

Different directions of the concentration gradients of different substances indicate that the formation of the bronchial fluid cannot be explained as a result of one factor only The composition of the tracheobronchial fluid resembles that of extracellular fluid. Too little is known of the foetal pulmonary capillary flow to describe the possible transport of water and other substances between blood and peripheral airways.

During the first breath the lungs are aerated explosively Tracheobronchial fluid disappears simultaneously even in tracheotomized ani[illegible] One might attribute this as to absorp[illegible] of the fluid into the pulmonary vessels

## Summary

Glucose, cholesterol and creatinine concentrations were determined in th amniotic fluid, foetal tracheobron hi l fluid and the blood of the mother anim l and foetus in the guinea pig and the sheep The foll w ing relative concentrations were observed

*Glucose* Guinea pig maternal blood foetal blood > amniotic fluid > tracheobronchial fluid. Sheep amniotic fluid > maternal blood ≃ foetal blood > tracheobronchial fluid.

*Cholesterol* Guinea pig foetal serum > maternal serum > amniotic fluid = tracheobronchial fluid. Sheep maternal serum > foetal serum ≃ amniotic fluid > tracheobronchial fluid.

*Creatinine* Guinea pig with smaller foetuses amniotic fluid > tracheobronchial fluid > foetal serum > maternal serum, guinea pig with larger foetuses: amniotic fluid = foetal serum > tracheobronchial fluid ≃ maternal serum all fluids: animals with smaller foetuses > animals with larger foetuses. Sheep amniotic fluid > tracheobronchial fluid = foetal serum > maternal serum

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# Hemolytic Uremic (Nephropathic) Syndrome

## *A Clinical Picture Unrecognized in the Past?*

by GJERMUND FLUGE and PETER JOHAN MOE

In 1955 Gasser *et al.* [2] published 5 cases of a disorder consisting of hemolytic anemia, thrombocytopenia and nephropathy which they named hemolytic-uremic syndrome. It occurred in infants and small children, all of whom died, and necropsy revealed renal cortical necrosis.

Piel & Phibbs [10] have collected 152 cases of this disorder from the world literature up to November 1965 61 of the patients died (40 per cent).

The onset of this disorder is usually characterized by mild to moderate gastroenteritis or upper respiratory infection, followed by pallor lethargy petechiae frequent ecchymoses hematuria, gastrointestinal bleedings, oliguria (anuria). Convulsions, hypertension and circulatory collapse are frequent in the more severe cases. Blood findings include hemolytic anemia, which may be mild or severe, thrombocytopenia and red cell fragmentation (distorted, irregular contracted cells, Burr cells and schistocytes) There is a marked variability in the pathologic changes including vascular lesions diagnosed as thrombocytopenic purpura renal cortical necrosis and acute glomerulonephritis

In 1966 MacLean and co-workers [9] published ten cases of this syndrome occurring in a small geographic area in North Wales over a short period of time. Definite thrombocytopenia was not present in any of these patients and only one of them died. The authors suggested that this syndrome, particularly in its milder forms, has formerly escaped recognition and is probably more common than the number of published cases indicates.

In order to see if it could be determined in retrospect whether hemolytic uremic syndrome had occurred previously without being recognized all records on patients classified as acute glomerulonephritis at the Children's Hospital, University of Bergen, during the 6-year periods 1950-1955 and 1960-1965 have been reviewed. The total material consists of 169 patients (104 during the first period) 56 per cent males and 44 per cent females. Fig 1 shows age distribution in both periods. It can be seen that 5.3 per cent of the patients were less than 2 years old. Two of these patients will be presented more closely

Case 1 L D 1561/53 a 5-year-old boy became ill 16 days prior to admission with facial edema, and exanthema of short dura

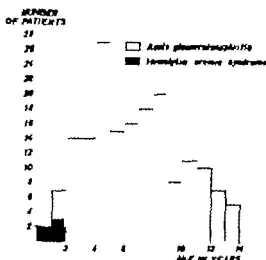


Fig 1 Age distribution in 167 cases of acute glomerulonephritis and 5 cases of hemolytic-uremic syndrome.

tion. Vomiting ensued and the urine was noted to be bloodstained. He was admitted to a peripheral hospital on November 22nd, 1953, with severe anemia (hemoglobin 28 per cent). His nonprotein nitrogen was markedly increased (228 mg per 100 ml) Purpuric lesions were noted on his skin and he also had hematemesis and hematuria. He received 3 blood transfusions and was transferred to the Children's Hospital, University of Bergen, on November 27th, 1953.

On admission the child was markedly pale and drowsy but was not distressed Temperature was normal, blood pressure 105/90 mm Hg There were petechiae and bruises on his skin but no edema.

The hemoglobin concentration was 10 1 g per 100 ml reticulocyte count, 6 per cent; platelets, 130,000 per mm³ white blood cells, 6200 per mm³ Coombs test was negative A peripheral blood smear (revised in December 1968) showed polychromasia with some fragmentation of the erythrocytes, reduced number of platelets. N definit erythroid hyperplasia was seen in the bone marrow

After admission, there were repeated falls in hemoglobin concentration despit several blood transfusions. Blood pressure increased to 170/100 mm Hg Platelet count dropped to 96,000 per mm³ and more purpuric lesions appeared on his skin Diuresis decreased and he was anuric and unconscious during the last days of his life. He died on the 1 th day of hospitalization. Autopsy was not permitted.

Case 2. E. J., 278/53, a 2 year-old girl was transferred from another hospital on December 24th, 1953, because of vomiting and abdominal pain of 6 days duration with hematuria the last two days.

On admission she was pale and drowsy Several bruises and petechiae were seen on the face and trunk. The temperature was normal, blood pressure 105/65 mm Hg The rest of the physical examination revealed nothing abnormal. The hemoglobin concentration was 4 6 g per 100 ml, reticulocytes, 8 per cent; platelets, 52,000 per mm ; white blood cells, 14,500 per mm osmotic fragility normal; blood non protein nitrogen 310 mg per 100 ml. Descriptions of peripheral blood and bone marrow smears are lacking. The bleeding, clotting and prothrombin times were normal. Throat cultures were negative urine culture grew E. coli. Antistreptolysin titres were normal. The urine contained albumin and microscopically numerous erythrocytes and granular casts, and the stools occult blood. She was treated with blood transfusions and improved temporary but one week after admission there was another episode of hemolytic activity with fall in hemoglobin and platelet count Thereafter there was a gradually improvement. The patient was discharged, after hospital stay of three months, with slight proteinuria. Follow up 1½ years later showed satisfactory general condition with normal blood pressure, urine and hemogram.

Two cases of hemolytic uremic syndrome were thus discovered on retrospective study of 104 patients classified as acute glomerulonephritis during the period 1950–1005 No patients with this syndrome were detected among the 65 cases treated for acute glomerulonephritis during the years 1000–1005

TABLE 1 *Clinical course in the last six patients with hemolytic uremic syndrome*

| Case No. | Sex | Age at onset | Symptoms | Blood pressure (mm Hg) | Treatment | End results |
|---|---|---|---|---|---|---|
| 3 | Male | 13 months | Persistent diarrhoea, with blood streaks, vomiting, oliguria, and muscular twitchings | 170/100 | Simple blood transfusions, hemodialysis | Complete recovery (follow-up 3 years after onset of illness) |
| 4 | Male | 16 months | Fever pallor weakness, purpuric lesions, anuria, and hepato-splenomegaly | 130/90 | Simple blood transfusions, Prednison 40 mg daily | Complete hematologic remission but bone lesions later on (acute reticulo-endotheliosis) |
| 5 | Male | 2 months | Continued vomiting and bloodstained loose stools, convulsions, and anuria | — | Simple and exchange transfusions. Peritoneal dialysis twice | Died 15 days after admission |
| 6 | Female | 4½ years | Tonsillitis, loose stools, gross hematuria, and pallor | 110/70 | Penicillin, simple blood transfusions | Complete recovery (follow up 3 months after onset of illness) |
| 7 | Male | 22 months | Persistent diarrhoea and vomiting, purpuric lesions, pallor and oliguria | 95/90 | Simple and exchange transfusions. Peritoneal dialysis. Heparin 10,000 units per sq m per day | Complete recovery except for slight proteinuria |
| 8 | Male | 1 month | High fever respiratory tract infection, diarrhoea and hemoglobinuria. Collapsed twice | — | Antibiotics, simple blood transfusions | Complete recovery (follow-up 2 months after onset of illness) |

Gastrointestinal symptoms are often seen in the prodromal stage of this disorder We therefore decided to review the records on patients dying from acute gastroenteritis during the two 6-year periods, 1950–55 and 1960–65 a total of 13 patients with a mean age of 10.5 months. The material is incomplete with respect to retrospective evaluation but the available data do not suggest the possibility of hemolytic-uremic syndrome in any of these patients

In addition to the two reported cases 6 patients with hemolytic uremic syndrome have been treated in the Children s Hospital in the period 1963–1966 Our material therefore, consists of 8 cases of hemolytic uremic syndrome two previously unrecognized cases from 1053 and 6 cases during the last 4 years Fig 1 also shows the age distribution of these patients. All of them were under 6 years, 5 less than 2 years old. There was no evidence of epidemic outbreaks and no seasonal accumulation was noted

The clinical course in the last 6 cases is shown in Table 1 T ble sh w some laboratory d ta in all the 8 cases with hemolytic-uremic syndrome

Necropsy in case 5 revealed both kidneys to be enlarged and swollen. There were no signs of bleeding or necrosis

TABLE 2. *Some laboratory data in the patients with hemolytic*

| Case no. | Hemoglobin (g per 100 ml) | Reticulocytes per 1000 | Platelets per mm³ | White blood cells per mm³ | Haptoglobin (mg per 100 ml) | Nonprotein nitrogen (mg per 100 ml) | Serum potassium (mEq per liter) | ABT units | Direct Coombs test | Fragmentation of erythrocytes |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7 4 | 72 | 96,000 | 6,400 | — | 229 | 4 1 | 220 | Neg | + |
| 2 | 4.6 | 80 | 52,000 | 14,500 | — | 310 | 5.. | 110 | Neg | Not examined |
| 3 | 4 1 | 30 | 73,000 | 17,300 | — | 373 | 5.5 | 30 | Neg. | Not examined |
| 4 | 3.. | 148 | 74,000 | 35,600 | — | 46 | 5.4 | — | Pos. | + |
| 5 | 4 4 | [illegible]8 | 140,000 | 23,600 | — | 720 | 5.9 | — | Not examined | + |
| 6 | 5.0 | 6 | 48,000 | 16,200 | 0 | 26 | — | 20 | Neg. | + |
| 7 | 4.8 | 43 | 56,000 | 8,500 | 0 | 550 | 6.6 | 100 | Neg | + |
| 8 | 8.6 | 106 | 10,000 | 9 700 | 31 | 164 | 4.2 | — | Not examined | – |

Histological examination showed diffuse changes as seen in fulminant acute glomerulonephritis. Special staining for fibrin thrombi was negative.

## Discussion

Retrospective analysis of the cases of acute glomerulonephritis demonstrates that the hemolytic-uremic syndrome must have occurred previously without being recog nized Cases 1 and 2 were both considered to be acute glomerulonephritis with toxic or allergic precipitated hemolytic anemia. No attention was paid then to the throm bocytopenia, and the fragmentation of the erythrocytes in case 1 has been taken as an artefact. The clinical course in these two cases is similar to that seen in hemolytic-uremic syndrome and both cases would be classified as such to-day

Patients no 1 and 2 resemble the three infants reported by Lamvik [7] as acute glomerulonephritis with hemolytic anemia in his review of 68 patients (from 1946 to 1960) with the final histological diagnosis of acute glomerulonephritis.

No cases of hemolytic-uremic syndrome were discovered among the patients dying from acute gastroenteritis. Perhaps an analysis of all cases of acute gastroenteritis might have been more inform ative in this respect.

This material throws no light upon etiological and pathogenic factors. Virological studies were not performed, nor special evaluation of coagulation factors and thrombocytes

Bacterial cultures of stools grew E. coli in patient no. 5 and E. coli and Proteus Morgagni in case no 7 Patient no. . had pyuria and E coli was detected in the urine. Throat cultures revealed nothing

*uremic syndrome*

| Bone marrow smears | Protein uria | Hemat uria | Granular casts |
|---|---|---|---|
| No definite erythroid hyperplasia | + + + + | + | + |
| Lacking | + + | + | + |
| Lacking | + | + | + |
| Erythroid hyperplasia | Transient | + | Not present |
| Erythroid hyperplasia | + + | + | + |
| Erythroid hyperplasia | + + | + | + |
| Erythroid hyperplasia | + | + | Not present |
| Normal | + + | + | Not present |

of significance and antistreptolysin titres were normal

The direct Coombs test was positive only in the infant with acute reticuloendotheliosis. With the exception of the case reported by Gasser *et al* [2] the test was negative in previously published cases

The inclusion of case 4 may be questionable, but his acute illness coincided with the onset of the underlying disease which later on turned out to be acute reticuloendotheliosis. This infant demonstrates that the *triad hemolytic anemia, thrombocytopenia and nephropathia* may be symptomatic of a basal disorder

Involvement of the central nervous system is common to most report on the hemolytic uremic syndrome but has not been a prominent feature in this material General convulsions were observed during dialysis in three patients, but were probably due to rapid changes in fluid and electrolyte equilibrium Apart from this only one patient had convulsions.

Uremia was not a constant finding in the cases reported as hemolytic uremic syndrome Of the ten patients reported by McLean *et al.* [9] only four had a blood urea concentration above 100 mg per 100 ml. In this study case no 6 had normal blood nonprotein nitrogen The course in this patient was much like that seen in acute glomerulonephritis and she would definitely have been classified as such previously The criteria for hemolytic uremic syndrome were however present. Serum haptoglobin determination seems to be of value in cases like this one, as it is reported to be high in acute glomerulonephritis [5] while a value of 0 mg per 100 ml was observed in our case As uremia needs not be present, the term hemolytic-nephropathic syndrome seems preferable

McKay [8] has suggested that the hemolytic uremic syndrome represents a state of hypersensitivity equivalent to the Sanarelli-Shwartzman's reaction [1*] in experimental animals. The theory that intravascular coagulation plays a primary role in the pathogenesis of this syndrome provides a rationale for anticoagulant therapy [6 11] Heparinization has also proved effective in other states of hyper coagulability like thrombotic thrombocytopenic purpura and purpura *fulminans* [1 4]. It seems important that the therapy should be instituted as early as possible to prevent renal cortical necrosis. Only one of our patient received heparin therapy (case 7) The effect of this treatment is difficult to evaluate as he also received exchange transfusion and peritoneal dialysis However he had no further bleed

ings and made a remarkable recovery in spite of his critical condition

Corticosteroid therapy was only given to patient no 4 who developed anuria while receiving this therapy

Only two of the eight patients died in spite of the fact that seven of them had marked azotemia. The oligonuric phase lasted longest in these two cases, 0 and 12 days respectively and both had hypertension.

Follow up examination seems to indicate that the prognosis is good if the patient survives the acute phase of this syndrome.

## Summary

Two cases of hemolytic-uremic syndrome were discovered on retrospective study of 104 patients classified as acute glomerulonephritis during the years 1050–105_ In addition six patients with hemolytic-uremic syndrome diagnosed during the period 1963–1966 are presented. Early diagnosis and the probable beneficial effect of anticoagulant therapy are stressed.

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# Natural History of Radiological Changes of Knee Joint in Juvenile Rheumatoid Arthritis

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Several authors [1–5] have presented evidence on the general aspects of radiological changes in different joints of juvenile rheumatoid arthritis. Sairanen [6], dealing with changes in the knee joint in juvenile rheumatoid arthritis, states that narrowing of the joint space is one of the earliest radiological signs, whereas erosive changes occur pproximately two years after the onset of the disease. According to Sairanen the late changes are: ankylosis, osteophytosis, subluxation of the tibiae and excessive growth of the diseased bones. Groekoest [5] points to the narrowing of the joint space caused by loss of cartilage in juvenile rheumatoid arthritis. Laaksonen [6] reports the onset of clinical involvement of rheumatoid arthritis in the age group under six years as occurring mainly in the knee joint but does not include x ray findings of the knee joint in the investigation.

It is customary [7 9] in classifying the radiological evidence of adult rheumatoid arthritis to include soft tissue swelling osteoporosis, narrowing of joint space bone erosions, periosteal opposition of bone deformities and ankylosis. These criteria cannot be applied as such to juvenile rheumatoid arthritis. This is due to the fact that the joint is growing, it has a greater cartilaginous component than the adult joint and is more complex by the presence of the epiphyseal plate and growth nuclei.

It is proposed in the present study to describe the natural history of knee involvement in juvenile rheumatoid arthritis in the light of radiological findings.

## Material

The material consists of 18 cases of juvenile rheumatoid arthritis with the onset of the disease beginning in one knee joint i.e. monarthritis, and followed up from 2–17 years, mean 8 years, with the help of some 296 radiographs (a–p view on one and lateral views on two films)

## Results

There were 13 cases in the age group with the onset before the age of six years. In all these cases the disease later spread to peripheral joints (fingers, toes, wrists an ankles) within two to three years.

In th group over six years there were five cases. Two of these five cases presented a monoarticular course during the first two to three years. The disease process in this age group showed a better prognosis in general when compared with those with the onset before six years. This is




Fig 1 The earliest changes in the knee joints of a four year-old girl. Soft tissue swelling on both sides. Beginning osteoporosis Larger epiphyses of tibia and fibula on the left side

reflected in the radiographs which reveal greater deformity as a result of the faster rate of growth in these younger bones. In one case belonging to the older age group there had been a monarthritis in one of the knee joints lasting over three years and showing severe pathological changes in the synovial biopsy specimen but without any radiological changes demonstrable in the knee joint This illustrates the difficulties in interpreting the findings.

TABLE 1 *The time of the appearance of x-ray changes following the onset of juvenile rheumatoid arthritis in the knee joint*

| | |
|---|---|
| *Stage 1* | |
| Soft tissue swelling Osteoporosis. Widening of joint space Narrowing of joint space | 2–3 months |
| *Stage 2* | |
| Additional t that in stage 1 Asymmetry of growth nuclei | 4–6 months |
| *Stage 3* | |
| Additional t that in stage 2. Dislocations | 7 12 months |
| *Stage 4* | |
| Additional to that in stage 3: Erosions. Ankylosis. | *3 years* |

The early diagnosis of juvenile rheumatoid arthritis is difficult especially in monarticular or oligarticular forms of the disease Trauma, tuberculosis, other infections and disturbed ossification patterns are the main additional differential diagnoses [10] Three out of 18 cases in this material had trauma to the affected joint at about the time of onset. Four other patients had been treated as tuberculosis prior to the final dignosis

The earliest radiological sign of juvenile rheumatoid arthritis in the knee joint is soft tissue swelling It appears during the first * 3 months (stage 1 in Table 1 Fig 1). During this same period osteoporosis can be seen. In one case narrowing and in two cases widening of the joint space could be found during this initial stage of the disease The interpretation of irregularity at the margins of the epiphyseal plates is



Fig. 2. Erosions in the intercondylar eminentia. Joint spaces are narrowed. The erosionlike changes on both sides of the left tibial condyle may even be secondary osteophytosis.

difficult. In three cases it was possible to follow the development of erosions and destruction of femoral condyles in this particular area with roughness in the early (* 3 months) stage of the disease (case in Fig. 1). On the other hand, similar roughness was found in the other knees which never have shown any signs of rheumatoid arthritis. It was even impossible to assess the asymmetricity of the roughness so that it might be of diagnostic or prognostic value

The following findings in addition to those mentioned above ppeared during 4 to 6 months following the onset (stage 2 in Table 1)· Asymmetric epiphyseal nuclei in three cases. Narrowing of joint space in two cases. Soft tissue swelling and osteoporosis in 14 cases, in two cases these changes were more pronounced in the knee first involved. Four children did not show any radiological evidence of disease during 5–6 months after the onset despite clinical signs of active rheumatoid disease in the knee joints.

During the 6- to 12 month period following the onset of the disease the following changes, in addition to those mentioned above, were found (stage 3 in Table 1) Valgus deformity of the knee joints in two cases.

In one case indistinct deformity of the intercondylar eminentia was to be found one year after the onset. This progressed during the next year to definite erosions (Fig *) This late appearance of erosions in intercondylar area is contradictory to earlier statements that this is an early sign of rheumatoid arthritis in the juvenile knee joint [9]

The first erosions were found two to three years after th onset in the respective joint (stage 4 in Table 1) This was noted in only three joints Accordingly an early diagnosis cannot be based on erosions The sit of the earliest erosions was in the intercondylar area and at the epiphyseal margins

When the cases had been followed up for four to five years all the knee joints



Fig. 3. Soft tissue swelling. Cortical decalcification. Erosions on the epiphysis of femur. Subluxation of tibia.

showed narrowing of the joint spaces, osteoporosis and valgus deformity. In one case there was subluxation of the tibia and the fibula with new formation of bone and coarseness of the trabecular pattern, especially in the femoral condyles (Figs. 3 and 4).

Secondary osteoarthritic changes were found in only two cases. This occurred 11 years after the onset.

Ankylosis was found in four cases. Two cases were ankylosed clinically but not radiologically. These cases may be interpreted as representing fibrous ankylosis.

In Table 2 typical radiological findings of juvenile rheumatoid arthritis compared



Fig. 4. Heavy osteoporosis and coarseness of the trabecular system. Needlelike, curved tibias and fibulas.

with those of adult rheumatoid arthritis are presented. The findings in juvenile rheumatoid arthritis are based on material

TABLE 2. *Comparison of x ray findings in adult (Soila [9]) and juvenile rheumatoid arthritis of the knee joint*

| | Adult | Juvenile |
|---|---|---|
| Soft tissue swelling | + | + + |
| Osteoporosis | + + | + |
| Changes in joint spaces | + + | + + |
| Narrowing of the joint space | + + | + |
| Widening of the joint space | + | + + + |
| Differences in the size of growth nuclei | | + + + |
| Periosteal changes | + + | + + |
| Changes in ossification | − | + + |
| Erosions | + + | + |
| Dislocations | + + | + + + |
| Ankylosis | + | + + |
| Secondary osteoarthrosis | + + | + |

+ + + = Typical, often to be found.
+ + = Relatively often to be found.
+ = Occasionally to be found.

presented in this paper while those of the adult form of the disease are based on the paper of Soila [9]. The main differences are due to the growing structures of the juvenile knee joint In addition, more soft tissue swelling, less erosive changes, more ankylosis and less osteoarthrosis were present in juvenile knee joints.

## Discussion

In juvenile knee joints irregularity of the articular surfaces of the bones, especially in the femoral head is thought to provide some information of earlyrheumatoid activity This, however was not the case

The radiological evidence of knee joint involvement in juvenile rheumatoid arthritis does not have the same diagnostic and prognostic value as those of the fingers, feet and cervical spine. This is due to the fact that radiological changes occur much later in the knee (definite changes occur two to three years after the onset) than in peripheral joints.

On the other hand, radiological changes in the juvenile kn joint rarely give a clue of the stage of the disease because there is a large discrepancy between the clinical and radiological findings.


## Summary

The material presented in thi paper is based on a study of the knees of 18 patients suffering with juvenile rheumatoid arthritis with a monarticular onset of the disease in knee joint. The cases were followed with repeated x rays during over a period of 2 to 17 years (mean 8 years)

The disease process in the age group under six years appeared to be more severe than in older children.

The radiological changes which were found were Soft tissue swelling, osteoporosis and widening or narrowing of the joint space within ?-3 months. Asymmetry of the growth nuclei in 4–6 months. Dislocations in 7–12 months. Erosions, ankylosis, in –3 years.

A comparison of radiological changes in juvenile and adult rheumatoid arthritis in the knee joint is presented.


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CASE REPORT

# The Syndrome of Congenital Pancreatic Insufficiency, Chronic Respiratory Disease and Chronic Liver Damage

by DANA HAVLÍKOVÁ, OTTO VYCHYTIL and JAN JELÍNEK

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Two siblings have been observed whose clinical picture was characterised by pancreatic insufficiency liver insufficiency and chronic respiratory disease The repeated sweat test and the course of the respiratory disease excluded the diagnosis of fibrocystic disease of the pancreas. Case histories and the pertinent laboratory investigations of these two patients form the basis of this record.

## Case Reports

*Case 1*

H S a girl now 25 months of age was born prematurely after an injury to the mother in the 30th week of an otherwise normal pregnancy Her weight was 1800 g Both parents are healthy

After the delivery she was dyspneic her chest was narrow and there was a marked abdominal enlargement. Her stools were offensive but not frequent and she failed to thrive

At two months of age she became cyanotic and developed a cough. X rays of the chest showed small patches of increased density scattered through both lungs and bilateral emphysema. She received broad spectrum antibiotics, plasma and blood transfusions, vitamins and her general condition improved. She was discharged at the age of ½ months and kept under observation in the out patient department, the subsequent progress of the child has been one of very slow improvement.

At five months of age she was admitted to our clinic on account of persistent dyspnea and anorexia. A physical examination showed her to be poorly nourished, afebrile cyanotic and dyspneic with intercostal retractions. The chest was surprisingly narrow the abdomen enlarged, there was marked hepatosplenomegaly The examination of lungs showed scattered râles on both sides. Her stools were bulky and foul in odour

Laboratory studies revealed pancreatic insufficiency and abnormal liver function tests. The haemoglobin was 80 %, red cells 4 6 millions/mm³ W.B.C 9700/mm with polymorphonuclears 42 %, lymphocytes 53 %. The sweat test was normal (chlorides 46.1 mEq/l) Urinalysis gave normal results. The roentgen changes were the same as described 2½ months earlier: bilateral scattered patches of increased density generalized emphysema.

Therapy with antibiotics, pancreatin, methyltestosterone, prednison vitamins B failed to improve the patient's general condition. She gained weight very slowly her growth was unsatisfactory and she was mentally retarded, She remained dyspneic showed intercostal retractions, but there was no cough and no auscultatory findings. She had malodorous stools. Her chest remained narrow and abdomen protuberant and there was still hepatosplenomegaly

TABLE 1 *Liver function tests Case 1*

| Date | Transaminases | | Kathepsin | Bromsul falein retention |
|---|---|---|---|---|
| | GOT | GPT | | |
| | (King units) | | | |
| VII.65 | 276 | > 400 | | |
| VIII 65 | 236 | 184 | | 8.5 – |
| XII 65 | 263 | > 400 | | |
| III.66 | 222 | > 400 | 4.1 index | |
| II 67 | 133 | 184 | 2.0 index | |

Repeated laboratory studies failed to reveal neutropenia, liver function tests remained abnormal, roentgenograms of the chest revealed identical changes as on admission. Repeated sweat test was normal (Cl 20.6 mEq/l) In the duodenal content no activity was demonstrable for pancreatic trypsin, lipase and amylase. Serum proteins, electrophoresis of serum proteins, calcium, sodium, potassium, chloride levels, non protein nitrogen, oral glucose tolerance test, serum cholesterol, PBI, BWR, erythrocyte sedimentation rate, serological test for toxoplasmosis and examination for cytomegalic inclusion-bearing cells were all normal. Roentgenograms of the skeleton and intravenous pyelogram revealed no abnormalities.

She was readmitted to the hospital for re evaluation at the age of 13 months. Her weight was 4.7 kg and she was 59 cm long. The physical examination and the laboratory studies showed no change, except for the roentgenograms of the chest which showed considerable improvement of the roentgen findings in spit of marked dyspnea of the child.

At present the child is 23 months of age her weight is 7 0 kg (less than third percentile), her height is 69 cm (less than third percentile) and she is slightly hypotrophic Her mental development corresponds to the standard for the age of 12 months. She is still dyspnoic, shows intercostal retractions and circumoral cyanosis when crying, but has neither cough nor auscultatory findings. The liver and the spleen are about 3 cm under the lower costal margins, the abdomen is distended, the stools are malodorous.

The laboratory studies reveal still abnormal liver function test (Table 1), the roentgenograms of the chest are within normal range The hematological studies reveal no signs of neutropenia leukopenia or marked anemia. There is glucosuria, the urinary excretion of amino acids is normal.

Case 2

O B., a boy now 12 months of age is the younger brother of the patient 1 He was born at term after a normal pregnancy and he weighed 2.7 kg Hepatosplenomegaly narrow chest, dyspnea and protuberant abdomen were present at birth. The neonatal jaundice had lasted 4 weeks.

He was first seen by one of us (D H ) at the age of 14 days. Physical examination revealed a malnourished child with dyspnea, protuberant abdomen, hepatosplenomegaly His lungs were clear to auscultation, the stools were normal.

At the age of 1 months the stools became malodorous, but the child showed satisfactory weight gain. At the age 1 2 months all clinical signs including dyspnea were unchanged and X rays of the chest revealed scattered patches f increased density in all lobes and generalized emphysema.

During the next six months the clinical condition and the roentgenograms of the chest remained unchanged, although the child was treated with broad spectrum antibiotics and pancreatin. The laboratory studies revealed abnormal hepatic function test and low trypsin, amylase and lipase in the duodenal juice. Serum proteins, electrophoresis f serum proteins, serum lectrolytes, alkaline phosphatase levels, non protein nitrogen, serum holesterol, PBI, BWR erythrocyt sedimentation rat serological test for toxoplasmosis, examination of urine and saliva for cytomegalic inclusion-bearing cells, oral glucose t lerance test were all normal. The sweat test was normal as well (Cl 25 9 mEq/l) Repeated blood analyses revealed normal findings without leukopenia, neutropenia or marked anemia.

At the age of 1 months, his weight is 6.0 kg (less than third percentile) and his

TABLE 2 *Liver function tests Case 2*

| Date | Transaminases GOT (King units) | GPT | Kathepsin | Bromsulfalein retention |
|---|---|---|---|---|
| VI.66 | 158 | 158 | | |
| IX.66 | >400 | >400 | | |
| I.67 | 310 | 430 | 1.92 index | 15 % |
| II.67 | 280 | 280 | 1 69 index | |

height 63 cm (less than third percentile) He is still dyspneic and shows intercostal retractions, but has no auscultatory findings. The liver is palpable 5 cm below the right costal margin, the spleen is enlarged as well, the abdomen is distended. His stools are foul in odour and bulky

The liver function test remain abnormal (Table 2) and examination of the duodenal juice showed n trypsin and lipase; amylase was present only in traces. The urinalysis showed glucosuria, values and pattern of amino acids are normal. The roentgenograms of the chest show considerable improvement of the chronic pulmonary disease; only ex aggerated bronchovascular markings can be demonstrated.

The mental development of the patient corresponds to the standard for the age of 5 months.

## Discussion and Conclusions

The common features in this syndrome developed in two siblings, are congenital hepatosplenomegaly pancreatic insuffi ciency chronic liver damage chronic pul monary disease normal sweat chlorides, failure to thrive growth retardation and retarded motor and mental development.

The pancreatic insufficiency is limited to exocrine function, since both patients had normal glucose tolerance tests. The clinical signs of this insufficiency have been present since the first days of life in the

TABLE 3 *Hematologic data, Case 1*

| Age (months) | Dat | Whit blood cells per mm³ | Neutrophils (%) |
|---|---|---|---|
| 2 | IV 65 | 6000 | 46 |
| 2 | IV 65 | 6200 | 43 |
| 5 | VII.65 | 9700 | 40 |
| 6 | VIII.65 | 6600 | 43 |
| 7 | IX.65 | 8700 | 48 |
| 9 | XI.65 | 8900 | 66 |
| 10 | XI.65 | 8200 | 54 |
| 13 | III.66 | 7700 | 37 |
| 14 | IV 66 | 7900 | 28 |
| 19 | IX.66 | 7400 | 46 |

patient 1 and since two months in her brother and in laboratory it was demon strated at the age of 5 and 6 months respectively The history of both children and the *failure to thrive in early infancy* suggest that this may be a congenital disorder

The steatorrhoea was not a gross one and resembled that in cystic fibrosis of the pancreas The penetrant foul odour is its most striking feature.

Liver damage was demonstrated in both patients at the age of 4 months, but the marked hepatosplenomegaly at birth sug gests that this disorder may be congenital as well.

The clinical signs of pulmonary disease were noted at birth in both cases. The roentgenograms revealed identical roentgen changes at the age of 2 months. small patches of increased density scattered

TABLE 4. *Hematologic data, Case 2*

| Age (months) | Dat | White blood cells per mm³ | Neutrophils (%) |
|---|---|---|---|
| 2 | V 66 | 9300 | 36 |
| 3 | VI.66 | 8900 | 34 |
| 6 | IX.66 | 6800 | 61 |
| 11 | II 67 | 7800 | 39 |

through both lungs and generalized em physema. In both children the roentgen changes were resistant to antibiotic the rapy and disappeared spontaneously between the ninth and twelfth months of age but the patients are still dyspneic and cyanotic on exertion.

The presence of two cases in one family strongly suggests that this is a genetic disorder but the etiology remains obscure We considered that in view of the normal sweat test, the persistent hepatosplenomegaly and the course of the pulmonary disease a diagnosis of fibrocystic disease of the pancreas could not be sustained.

The cases described resemble to a certain extent congenital hypoplasia of exocrine pancreas [1 2 4 5, 6, 7]. None of the patients described in the literature showed abnormal liver function, but at necropsy fatty infiltration and/or cirrhosis of the liver were observed in some cases.

The chronic pulmonary disease with clinical signs developed at birth, has not been noted in congenital hypoplasia of the pancreas; some of the patients described had recurrent respiratory infections.

Shwachman *et al* [8] recorded a syndrome consisting of pancreatic insufficiency failure to thrive leukopenia, neu tropenia and anemia and intermittent galactosuria. Bone marrow xamination revealed hypoplasia of all elements in varying degrees. Bodian and his co-workers [3 4] conn that the congenital hypoplasia of exocri ancreas and the syndrome described b hwachman *et al* might be identical and t feel that hematological disturbances might be optional but not obligatory in this entity

Neither of our patients has had leukopenia, neutropenia or marked anemia so far

The clinical manifestations and the laboratory studies of our two patients suggest that they might represent an unrecognised variant of the congenital pancreatic lipomatosis with hypoplasia of exocrine elements. Unfortunately the clinical con ditions of both children do not allow of biopsy of the pancreas and therefore it has not yet been possible to obtain histological proof of the diagnosis.

## Summary

The case histories of two siblings are presented The cases represent a previously unknown clinical entity which is characterised by congenital pancreatic insufficiency chronic pulmonary disease chronic liver damage failure to thrive growth retardation and retarded motor and mental development The characteristic clinical pi ture was developed at birth.

The syndrome is unrelated to cystic fibrosis of the pancreas and a possible relation to congenital pancreatic lipoma tosis with hypoplasia of xocrine element is suggested

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CASE REPORT

# Mosaic Trisomy of an Autosome in the 6–12 Group in a Patient With Multiple Congenital Anomalies

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Three autosomal trisomies are known to be associated with well defined clinical syndromes. They are mongolism with 21 trisomy [10] 13–15 trisomy [12] and 17–18 trisomy [3].

An extra autosome among the chromosomes of the 6–1 group in all cells of the body seems in most instances to be lethal and has hitherto except in spontaneous abortions [2, 22], only been reported in a mentally retarded girl with multiple anomalies [7]. However chromosome studies were made only on peripheral blood cells.

Normal/6–12 trisomy mosaicism, on the other hand, i.e. the presence of a certain proportion of normal cells together with cells with 47 chromosomes, seems to have less drastic effects. To our knowledge 6 such cases of possible mosaic trisomy for a 6-12 autosome have been reported [4 6 13, 17 19 20 21]. Furthermore, a pre mature non-viable infant has been reported [15], who possessed two abnormal stemlines, one with an additional small chromosome and another with an additional autosome in group 6–12. We have observed a further case of mosaic trisomy for one of the autosomes in the 6–12 group in a mentally retarded boy with multiple anomalies, including agenesis of the corpus callosum.

## Case Record

### *Family history*

The parents, born in 1939, were both healthy There was no consanguinity either in the parents or in the maternal or paternal grandparents. A maternal first cousin of the patient was a high grade mental defective. Otherwise no instances of mental retardation, congenital malformations or other relevant conditions had been recorded among the near relatives of the patient.

The mother gave no history of abortions. She had had only two pregnancies; the first resulted in a normal girl, born in 1962, whose subsequent health and development had been normal. The second pregnancy resulting in the birth of the present patient, was uneventful.

### *Description of the patient*

L. J., a boy was born on December 3 1964, 18 days after term. The delivery was uneventful. His birth weight was 3290 g and height 50 cm. Feeding difficulties and hyper excitability were apparent already durin the newborn period. At 7 w ag boy was referred to the De o districs t the County H

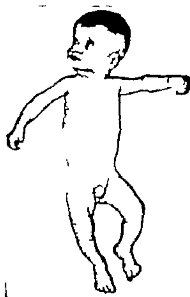


Fig. 1



Fig. 2 a



Fig. 2 b

Fig. 1. The patient at 6 months of age.
Fig. 2a and b. The patient at 2 years and 2 months of age

Physical examination revealed a peculiar appearance with a prominent forehead, short neck, large fontanels, large and broad nose, small palpebral fissures and a receding chin. There was generalized muscular hypertonia. The fingers were kept in a flexed position and were difficult to extend. The palmar skin was unusually thick and folded. The boy had a markedly shrill, monotonous and protracted cry.

H. was readmitted to the clinic at 3 months of age because of repeated upper respiratory infections and feeding difficulties. At that time his limbs showed generalized stiffness. A pneumoencephalogram was abnormal and consistent with agenesis of the corpus callosum. EEG was normal. A Gesell test at 17 weeks of age showed a developmental age of 1 weeks.

When 6 months of age (Fig. 1), the patient was admitted to the Department of Paediatrics, University Hospital, Uppsala, for further examination. His height was 66 cm (normal for his age), weight 6.6 kg (normal for the height) and head circumference 45 cm (about 0.5 cm less than $-2$ S.D. for height of 66 cm). The forehead was prominent and the surface of the skull bossed. The anterior fontanel was large with irregular margins. His ears were low-set and showed a dysplastic configuration. The palate was highly arched and narrow. The uvula was split. The nose was somewhat large and the chin small. The neck was short, but no webbing could be seen. The thumbs were set more proximally than normal and lacked ordinary abduction and extensibility. All fingers showed a moderate flexion contracture and the palmar skin was abnormally thick and folded into deep creases. There were no simian creases. The knee joints had an extension defect of 30°. The skin of the soles was thickened with deep furrows, including a plantar furrow running from the cleft between the first and second toes bilaterally (Fig. 3). The first toes were abnormally long and showed a medial deviation. On physical examination the heart was normal. The liver and spleen were not palpable. The penis and scrotum were normal. The right testis was not descended, the left could be felt in the scrotum. His psychomotor development was now clearly retarded.

Neurologic examination showed increased



Fig. 3. The feet of the patient showing abnormally long first toes and deep plantar furrows.

muscular tone of the legs and brisk tendon reflexes. The grasp reflex persisted. Arm protection and diving reactions could not be elicited. Ophthalmoscopic and electroencephalographic examinations were normal. The patient responded to sound.

Pneumoencephalography revealed a dilated third ventricle reaching higher up than usual between the slightly laterally displaced and relatively narrow lateral ventricles. The temporal horns were dilated. There was a close relation between the interhemispheric fissure and the roof of the third ventricle indicating considerable reduction of the corpus callosum. Conclusion: partial agenesis of the corpus callosum.

On reexamination at 36 months of age (Fig. 2a and b) his motor development was equivalent to that of a child of 12–18 months. He could not speak a single word and his social and adaptive development corresponded to a child of about 11 months. His height was 93 cm (normal for his age). The measurements from pubis to crown and pubis to sole were 41 and 52 cm respectively. The span was 85 cm. His weight was 12.2 kg (corresponding to −2 S.D. for height of 93 cm). Craniometry gave the following dimensions: circumference 53 cm, anteroposterior length 17.5 cm, width 14.2 cm and cephalic index 81. The anterior fontanel measured 1.5 × 1.5 cm. In addition to the features noted at 6 months of age the patient showed an elongated and slender trunk with relatively short legs (Fig. 2b). The ability to extend the finger and knee joints was still limited. There was a moderate flexion contracture of the hip joints and limitation of

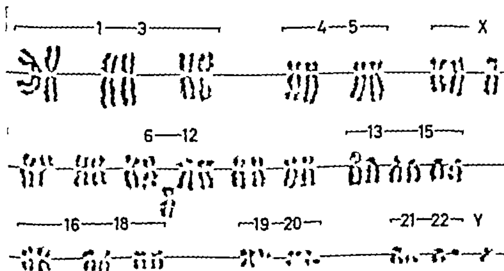



Fig. 4. A 47 chromosome karyotype including an extra chromosome in the 6–12 group

dorsal flexion in the right ankle joint. The extensor muscles of the hands were hypoplastic. Except for a moderate convergent squint of the left eye and brisk tendon reflexes, neurologic examination revealed no abnormalities. The right testis was still undescended and the left small and soft.

X ray films of the skeleton revealed a broad and flattened body of the first lumbar vertebra, open arches of L 1 and L 5, spondylolysis of the arch of L 4, increased interpeduncular distance of L 3, a rudimentary arch between L 2 and L 3 and coxa valga. The number of ossification centers corresponded to his age. The soft tissues of the legs appeared normal on X ray examination.

*Laboratory tests*

Routine tests on blood and urine were normal. The serum potassium, calcium, phosphorous, alkaline phosphatase and fasting blood glucose concentrations were normal. Wasserman and toxoplasma reactions were negative. Serum immunoglobulin determination according to the method of Mancini *et al.* [11] showed normal concentrations of IgG, IgA and IgM. The cerebrospinal fluid was normal (protein 35 mg per 100 ml). Two-dimensional paper chromatography of aminoacids in the urine gave a normal pattern. Urinary excretion of uronic acid was [illegible]0 mg/24 hours (normal).

## Cytological Observations

Chromosome studies were made in blood leucocytes and in cells derived from a skin biopsy obtained from the patient and grown *in vitro* and also in cultured leucocytes from his sister and parents. The blood as well as the skin cultures from the patient contained two different cell populations, one with 46 chromosome cells having a normal male karyotype and another with 47 chromosome cells (Fig. 4) including an extra chromosome in the 6-12 X group.

| | 46 | 47 | Total |
|---|---|---|---|
| Patient, blood (several cultures) | [illegible] | 60 | 120 |
| Patient, skin | 4 | 46 | 50 |
| Sister blood | 15 | — | 15 |
| Mother blood | 15 | — | 15 |
| Father blood | 15 | — | 15 |

Blood cultures from the patient were labelled with tritiated thymidine to examine the pattern of the DNA synthesis. Autoradiographs were prepared according to Schmid [16]. No late replicating X chromosome was observed. Buccal mucosa cells and cultured skin cells from the patient lacked sex chromatin. Of 500 polymorph leucocytes examined no drum stick was found. An average of 1.6 per cent of the mature neutrophils had two or more hook like appendages of nuclear chromatin of the type described by Powers *et al.* [14]. The parents and the sister of the patient had normal karyotypes.

## Discussion

The extra chromosome of group 6-12 X observed in about half of the cells in the blood and skin cultures of this patient could have been an autosome or alternatively an extra X chromosome. The autoradiographic results proved however that the extra chromosome was an autosome rather than an X chromosome. Moreover the negative sex chromatin pattern and the extensive physical malformations are not compatible with an XY/XXY Klinefelter syndrome.

Among the previously reported patients with this type of extra chromosome autoradiographic examinations were undertaken in two cases only [4, 16]. Because of the mosaicism characteristic of most of these cases, absence alone of sex chromatin cannot prove that the extra chromosome is of an autosomal nature. Auto-

radiography is required for differential identification.

The extra chromosome may have been included following somatic non-disjunction after the first cleavage division of a normal zygote or as a consequence of chromosome lagging during anaphase of an originally trisomic zygote

The six previously reported patients with trisomy 6–12 mosaicism displayed a variety of clinical signs and symptoms However four were mentally retarded and four had genital anomalies. One of the patients a woman, was described as men tally and physically normal [ 1] This patient and a patient with primary amenorrhoea reported by Jacobs *et al* [6] both had a higher proportion of normal cells than the remaining four patients.

The variable clinical manifestations in these patients may of course be referred etiologically to different types of 6–1? trisomies and modified by varying proportions of normal and trisomic cells in individual patients.

Some of the clinical manifestations observed in our patient appear similar to those of a patient reported by Pfeiffer *et al.* [13] i.e. mental retardation muscular stiffness, articular extension defects abnormally shaped skull, low-set dysplastic ears receding jaw long and slender trunk vertebral anomalies and cryptorchidism. Jalbert *et al* [7] described a girl with an extra autosome of the 6–1 group consistently found in all cells from blood cultures She also showed similar signs and symptoms to those of our patient viz. mental ret rdation, low-set nd dysplastic ears receding hin long and slender trunk, extension defects of the finger joints and restricted mobility of th ankle joints However similar m.nifestations are oft seen in association w h a variety of autosomal aberrations.

Another patient des ibed by Stalder *et al.* [10 20], also resembles our patient inasmuch as he was ment lly retarded, had restricted mobility of the joints marked plantar creases coxa alga abnormalities of the lumbar vertebrae convergent strabismus and agenesis of the corpus callosum. By contrast howeve this patient showed increased height-age and advanced skeletal age Nevertheless the similarities of clinical manifestations between these two patients are extensive enough to suggest also karyotypic simi larity Further investigations are required to ascertain whether they represent an identifiable specific trisomy of th 6–1 chromosome group.

The absence of the corpus callosum in these two patients is of particular interest This defect has been observed in association with the 13–15 trisomy syndrome [1 5 8, 9 18] but not with other known chromosomal syndromes Such defects are probably caused by failure of the prosencephalon to divide during early em bryogenesis. On complete failure this leaves a forebrain with a single ventricle la king a corpus callosum as well as a septum pellucidum. Incomplete separation of the anterior parts of the hemispheres will cause less severe defects, i.e partial or total agenesis of the corpus callosum The pneumoencephalographic findings in our patient are consistent with this latter less severe form of prosencephalic defect

## Summary

A congenital malformation syndrome including genesis of the corpus callosum

and mental retardation is associated with the presence of two different cell populations one with 46 chromosome cells having a normal male karyotype and another with 47 chromosome cells including an extra chromosome in the 6–12 group (the X chromosome was excluded by autoradiography) The proportion of the two cell lines is about 1 1

## Acknowledgement

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CASE REPORT

# Hereditary Ectodermal Dysplasia of Anhidrotic Type with Increased Protein Bound Serum Thyroxine

by ERIK HIPPE

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The triad of incomplete development of the sweat glands, defects of the tooth buds and scanty hair growth is characteristic of anhidrotic ectodermal dysplasia. According to Darwin [6], the first description of the disease was given in 1838 by Wedderburn, who reported a Hindu family presenting symptoms of the disease. Cockayne [5] collected a great number of various ectodermal defects, but the most typical feature of the disease described here is the anhidrosis, resulting in the most descriptive term, anhidrotic ectodermal dysplasia, suggested by Weech [20] in 1929 which is now universally accepted. Almost 300 cases are on record in the literature of which Perabo *et al* [15] in 1956 and Jespersen [12] in 1962 shall be mentioned as good review articles accompanied by reports of cases observed by themselves.

Occasionally combination of other congenital malformations has been reported in the same patient and also cases of ectodermal anhidrotic dysplasia associated with mesodermal defects. Lack of mucous secretion in the respiratory tract was described by Perlman [16] and de Jager [11]. I patient with anhidrotic ectodermal dysplasia who died from pneumonia, the latter author found total absence of mucous glands in the mouth, the pharynx, the upper part of the oesophagus, and the respiratory tract. Another variant of anhidrotic ectodermal dysplasia has been described, associated with polydactyly, chondrodysplasia, and congenital heart disease (Ellis-van Creveld syndrome [7]).

In 1930, Thannhauser [10] described a patient with anhidrotic ectodermal dysplasia presenting symptoms of adrenal medullary insufficiency. In the following, a case will be reported which gave rise to the detection of the combination of familiar anhidrotic ectodermal dysplasia and increased protein-bound serum thyroxine in otherwise euthyroid individuals.

## Case Report

The patient was a boy 3 months old, who was admitted to the hospital with hyperthermia and convulsions. Pregnancy, delivery and neonatal period had been normal. The birth weight was 3030 g, length 51 cm. The patient had hitherto been well and had thrived and developed normally although he had often been hot, restless, and shaking. Two days before admission nasal obstruction was noticed. On the day of admission

he had attack of fever up to 41.6 C and trembling of the arms. On admission, the patient was found to be hot, flaccid and pale. He had a few attacks of dry cough without cyanosis. The nutritional state was adequate: weight 5830 g, height 61 cm. The face was peculiar, resembling old age. The head circumference was 40 cm, the frontal fontanelle measured 5 by 5 cm. The hair of the scalp was thin lanugo hair, the eyebrows were almost absent. Around the eyes there was reddish brown pigmentation, the nose was a little broad, but not saddle shaped. The lips were protruding with dry fissured prolabia. On the neck there were several small glands, no struma was present. The tonicity of the extremities was slightly increased but varied somewhat during examination. The deep reflexes were normal. The fingers were abnormally long. The patient could smile and fix his attention on objects and followed the surroundings with his eyes. The skin was very dry. Apart from a slight redness of the throat, the rest of the physical examination was unremarkable. During the succeeding days, the temperature varied considerably from normal to brief attacks of fever up to more than 40 C, during which the patient was restless, trembling with increased tonicity of the limbs and crying hoarsely, almost aphonically. The tonicity varied from normal to slightly increased, particularly during brief periods of extremely rapid and shallow breathing. The meals were often interrupted by attacks of dry coughing without expectoration or cyanosis. Following a possible aspiration during a meal, the patient developed pneumonia, during which he was seriously ill and bronchial irrigation and aspiration had to be carried out. On laryngoscopy it was observed that all mucous membranes were extremely dry. Long term treatment with tetracycline was instituted and, in the case of respiratory distress, inhalation treatment with atomized Alevaire[a]. The patient was discharged at the age of 5 months with this medication and with instruction to the parents as to the measures to be taken against hyperpyrexia. Since then the patient has thrived normally and, at a follow-up examination at the age of 9 months, the psychomotor development was found to be normal.

*Laboratory examinations during the hospital stay*

Erythrocyte sedimentation rate 13 mm/hour. Haemoglobin 11.1 g per 100 ml. MCV 6 mpl. MCHC 36 g per cent. Blood group: 0, Rh positive. WBC 7 600 per µl. Differential count: normal distribution of leucocytes. Serum electrophoresis: 4.53 albumin, 0.11 alpha 1 globulin, 0.65 alpha 2 globulin, 0.51 beta globulin, and 1.23 gamma globulin in g/100 ml of serum. Total protein 7.10 g/100 ml. Total serum cholesterol 207 mg/100 ml. Glucose tolerance test normal. Wassermann reaction negative. Toxoplasmosis test negative. Blood culture during attacks of fever showed no growth. Serum creatinine 0.5 mg/100 ml. Examination of urine: no albumin, sugar or acetone; microscopic examination normal; tests for amino acids and acid mucopolysaccharides normal. Urinary excretion of 17 ketogenic-steroids 1.1 mg/24 hours (within normal range for the age). The spinal fluid contained normal quantities of protein and sugar and no blood cells. EEG was normal.

The clinical picture suggested anhidrotic ectodermal dysplasia and consequently roentgen examination of the jaws was made (Fig. 1). In the upper jaw only three teeth were seen, two in the position of the canines and one as a right incisor. In the lower jaw there were only two teeth corresponding to the canines. All the teeth were atypical, pointed and cone-shaped, and it could not be determined whether they were deciduous or permanent teeth. This is in accordance with the findings in anhidrotic ectodermal dysplasia [10]. Skin biopsies from areas where hair and sweat glands should be found, showed complete absence of these structures.

Because of the patient's dry myxoedematoid skin, determination of protein-bound iodine and triiodothyronine ($T_3$) test were carried out (Table 1). The determinations revealed a markedly increased level of pro-

tein bound iodine and a low T test. Serum thyroxine (T) determined by applying modification [17] of the method described by Murphy [14] was markedly increased, whereas the dialysable fraction of thyroxine was relatively reduced. After administration of radioiodine normal uptake by the thyroid was found, whereas the radioactive protein bound iodine in serum was slightly elevated (0.5 % of the iodine administered per litre of serum). The serum did not contain thyroid antibodies, tested by a fluorescence technique. Clinically the patient in other respects was found to be euthyroid. Roentgen examination revealed normal development of ossification centres. Chromosome analysis was normal.

Hence, in addition to the patient's ectodermal dysplasia, a disorder of thyroid metabolism was found, characterised by an increase in protein-bound serum thyroxine. Since both disorders are known to occur as hereditary diseases, although separately, the patient's family was studied.

One of the patient's siblings, a brother aged 6 years, was perfectly healthy without any signs of ectodermal dysplasia or thyroid disorders. Serum thyroxine was slightly elevated but with a normal dialysable fraction. A sister had died at the age of 6 weeks from congenital heart disease (truncus arteriosus communis).

The father of the patient was healthy with no signs of ectodermal dysplasia, and his



Fig. 1 Radiograph showing dental status in a 3-month-old boy with anhidrotic ectodermal dysplasia. Lateral view with frontal views inserted.

blood contained normal quantities of thyroxine.

The mother had never had upper or lower incisors. Furthermore she had very thin hair and scanty lanugo hair on the [illegible]

TABLE 1 *Laboratory findings in the patient and some of his relatives with increased protein-bound iodine in serum*

| Subject | Age | PBI μg/100 ml (normal range 3.4–7.0) | Resin absorption of T (% of normal) | $T_4$ by the method of Murphy μg/100 ml (normal range 4.6–10.6) | Dialysable $T_4$ in % of total $T_4$ (normal range [illegible]) |
|---|---|---|---|---|---|
| Patient | 3 mos. | 13 1 14–8 | 23–33 | | |
| Mother | 23 yrs. | 10.4 | | 34.8 | [illegible] |
| Father | 24 yrs. | 8.7 | | 19.8 | |
| Brother | 6 yrs. | 7.1 | | 11.8 | [illegible] |
| Maternal grandmother | 54 yrs. | 9.8 | | 15.4 14.2 | [illegible] |

and no hair on the right. She was able to sweat normally. There were no clinical signs of thyroid disorders. The serum thyroxine was markedly increased with a reduced dialysable fraction.

The maternal grandmother had very thin hair with alopecia and absence of hair on arms and legs. Dental development and ability to sweat were normal, and no signs of thyroid disorders were present. In this case also increased serum thyroxine with a low free dialysable fraction was found.

The examinations made in the members of the family are listed in Table 1.

## Discussion

In three generations, we were able to demonstrate the presence of ectodermal anomalies, associated with increased levels of protein bound serum thyroxine.

In the literature anhidrotic ectodermal dysplasia is often reported to be a hereditary sex linked, recessive disorder [12], which is in accordance with the fact that more males than females are born with the disease. However in some families, as in the one reported here it seems to be an irregular dominant disorder [5] or more probably a manifestation of Lyon variability with a few symptoms in the mothers whereas the sons possess the characteristic disease.

In reviewing the literature it was found that of the cases reported, almost 300 only about 10 were diagnosed before the patients were 6 months old. No doubt this is due to the fact that during infancy attacks of fever may easily be explained by the presence of trivial infections, and that one of the most characteristic symptoms namely the dental abnormalities, cannot be recognized immediately. However an early diagnosis is extremely important since the infants may die from hyperpyrexia [4, 12], occasionally with respiratory infections [11] or with febrile convulsions. Furthermore, early odontological treatment is important [10], partly to preserve the few teeth present, partly with a view to the fitting of a prosthesis. Similar to the cases reported [11 16] the patient's dry cough, hoarse crying, and the laryngoscopical findings suggest a dysfunction, presumably absence of the mucous glands in the respiratory tract i.e. changes in tissues of mesodermal origin. Although the nervous system is an ectodermal structure, cases of anhidrotic ectodermal dysplasia involving this system have never been reported. Patients of normal [1] and low [9 13] intelligence have been described.

The finding of increased protein-bound iodine in the present family is not caused by iodine contamination, but by an increased level of serum thyroxine, however proteinbound to such an extent that the free active amount of thyroxine is normal. When, as in Table 1 the free fraction is calculated in per cent of the total amount of thyroxine a low percentage is found. Normally a somewhat higher percentage is found in the free stage, when the protein bound thyroxine is not elevated. The low $T_3$ test suggests a high binding capacity in the serum protein of triiodothyronine and thereby also of thyroxine [ ]. Administration of radioiodine showed normal uptake by the thyroid, whereas the radioactive protein bound iodine in serum was slightly increased as compared with normal levels after 24 hours—indicating perhaps a high turnover of thyroxine.

Familial increase in the thyroxine-binding globulin has been described previously

in two families, in 1950 by Beierwaltes & Robbins [ ] (further examined in 1961 by Beierwaltes *et al.* [3]) and in 1962 by Florsheim *et al.* [8], but it is a relatively rare finding. The hereditary pattern is described as dominant and not sex linked [3 8], which agrees with the findings in the family reported here

The combination of anhidrotic ectodermal dysplasia and increased serum thyroxine has not been described previously The question may be raised as to whether anhidrotic ectodermal dysplasia is always associated with increased serum thyroxine In one of the cases of anhidrotic ectodermal dysplasia reported by Jespersen [1*], in which the level of protein bound iodine is given this value was within the normal range By examining the 5 cases previously reported by Holst [10], Sierabæk Nielsen [18] found normal serum thyroxine Hence the combination of familial anhidrotic ectodermal dysplasia and increased protein bound serum thyroxine might be considered to be a coincidence or it might be a new syndrome

## Summary

A case of anhidrotic ectodermal dysplasia is reported in a boy 3 months old who was admitted because of hyperpyrexia Apart from the ectodermal changes, deficient mucous secretions of the glands in the respiratory tract and markedly increased protein-bound iodine were found. The patient's blood contained an increased amount of protein bound serum thyroxine but normal amounts of free thyroxine, which was in accordance with the fact that the patient was clinically euthyroid. In some relatives, ectodermal changes could be demonstrated and, in the blood, in*creased amounts of protein-bound serum* thyroxine were found in individuals otherwise euthyroid. Most likely this is a case of an hereditary increase in the thyroxine-binding globulin, coincidentally combined with ectodermal dysplasia.

## Acknowledgement

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# ANNOUNCEMENTS

## European Congress of Perinatal Medicine

The European Congress of Perinatal Medicine will be held in the Congress Hall, Berlin, 28th–30th March 1968.

*Provisional program*

Amniotic Fluid and the Early Detection of Foetal Hypoxia Prevention of Rhesus Iso-Immunization Antenatal Diagnosis and Treatment of Rhesus Incompatibility and the Immediate Treatment of Hydropic Infants The Clinical Significance of Electronic Methods for Monitoring the Foetal Heart Foetal Blood Sampling Important Clinical Relationship between the Mother and Foetus during Labour Resuscitation of the Newborn The High Risk Baby Modern Obstetrics in Relationship to Paediatrics Measures for Reducing the Perinatal Mortality Rate.

*Scientific Committee* P Huntingford, London, L. S. Prod'hom, Lausanne, B Vedra, Praha K. Fischer Hamburg E. Saling, Berlin. — For further information write to Hansisches Verlagskontor Abt. Kongress-Informationen, Address: Hansisches Verlagskontor H. Scheffler Lubeck, Brehmerstrasse 16 West Germany Applications to attend the Congress should be mad at the latest by 15th January 1968.

## American Cleft Palate Association

The American Cleft Palate Association announces its plans to sponsor an International Congress on the subject of Cleft Palate April 14 through April 17 1969 t the Shamrock Hilton Hotel in Houston, Texas. Further information relative to this Congress may be btained by writing to: Betty Jane McWilliams, Ph.D Cleft Palate Research Center The University of Pittsburgh, 320 Salk Hall Pittsburgh Pennsylvania 15213 U.S.A.